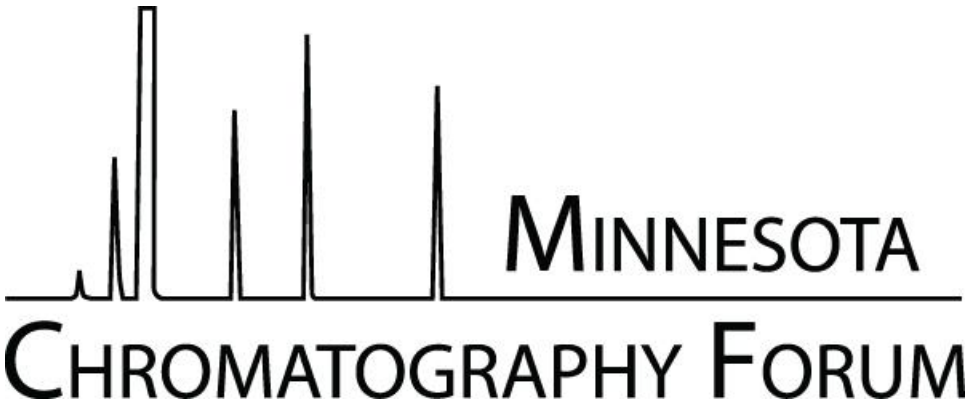

43rd Annual

MCF Spring Symposium

Heritage Center of Brooklyn Center

May 7-9, 2024

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Map of Heritage Center of Brooklyn Center

MCF SPRING SYMPOSIUM: May 7-9, 2024

Daily Program

Tuesday, May 7, 2024

7:30 AM	Registration & Check-In Breakfast Station & Beverages	Harvest Room (Door 4)
8:00 AM – 4:30 PM	Concurrent Short Courses "Optimizing GC Methods and Troubleshooting" "CSI MCF" "Troubleshooting LC and (U)HPLC Systems" (Starts at 8:30)	Harvest Rooms A, B, C
BREAKS:		
10:00 AM	Break (Snacks & Beverages)	Harvest Room
12:00 PM	Lunch	Garden City Ballroom
2:30 PM	Break (Snacks & Beverages)	Harvest Room

Wednesday, May 8, 2024

7:30 AM	Registration & Check-In Breakfast Station & Beverages	Harvest Room (Door 4)
8:00 AM – 12:00 PM	Concurrent Short Courses "Optimizing GC Methods and Troubleshooting" "CSI MCF" "Introduction to (U)HPLC" (8:30-2:00)	Harvest Rooms A, B, C
10:00 AM	Break (Snacks & Beverages)	Harvest Room
12:00 PM	Lunch	Garden City Ballroom
12:00 PM – 6:00 PM	Vendor Exhibits Open	Carriage Hall
2:30 PM – 5:00 PM	Vendor Seminars	Captain's Room
3:30 PM – 6:00 PM	Reception (Hosted wine/beer, hors d'oeuvres)	Carriage Hall

Please note: Registration is not required for the Vendor Exhibits, Vendor Seminars, and Reception on Wednesday.

Registration is required to attend Thursday's sessions

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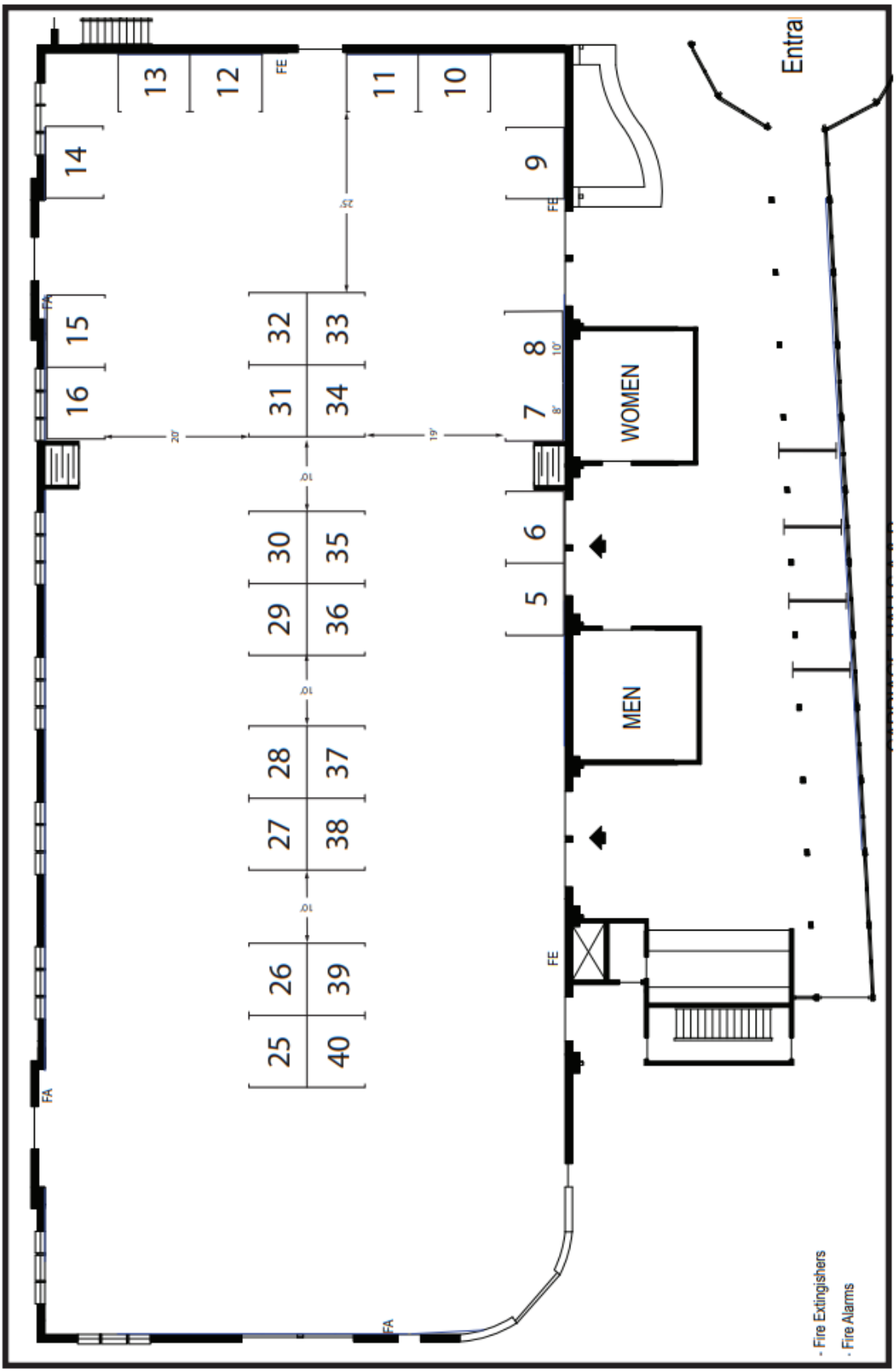
Thursday, May 9, 2024 (Registration Required)

8:00 AM	Registration Breakfast	Carriage Hall (Door 1) Garden City Ballroom
8:45 AM	Introduction	Garden City Ballroom
9:00 AM	Keynote Address "Multidimensional Chromatography Analyses as an Investigative Tool" by Dr. Katelynn Perrault Uptmor, William & Mary	Garden City Ballroom
10:00 AM	Morning Break; Exhibits Open & Poster Session	Carriage Hall & Atrium
10:30 AM – 12:00 PM	Morning Session: Sample Preparation and Environmental Analysis	Captain's Room (Main Level) See Page 10 for Full Program
12:00 PM – 1:30 PM	Lunch	Garden City Ballroom
12:00 PM – 2:00 PM	Exhibit Hall Open	Carriage Hall
1:30 PM – 2:40 PM	Afternoon Session: Stationary Phases and Mobile Phases	Captain's Room (Main Level) See Page 11 for Full Program
2:40 PM – 3:00 PM	Break	
3:00 PM – 4:00 PM	Afternoon Sessions: Novel Methodologies Pharmaceutical Analysis	Captain's Room (Main Level) Tack A/B (Lower Level) See Page 11 for Full Program
3:30 PM – 4:30 PM	Exhibit Hall Open	Carriage Hall
4:10 PM	Door Prize Drawing MCF Board Elections	Carriage Hall

Exhibitor Booths and Exhibit Floorplan (May 8 & 9)



2024 Symposium Exhibitor Location & Contact Information

Vendors Alphabetically with Booth Number

Agilent Technologies	37, 38
Aminoacids.com	10
Bruker	9
Chrom Tech, Inc.	7, 8
Cytiva	30
Fisher Scientific	15
GERSTEL, Inc.	34
Matheson	14
Metrohm USA	31
Pace Life Sciences	40
Peak Scientific	39
Quantum Analytics	35
Restek	5
Sciex	6
Shimadzu Scientific Instruments, Inc.	36
Thermo Fisher Scientific	16
Waters Corporation	32, 33

Vendors by Booth Number

Restek	5
Sciex	6
Chrom Tech, Inc.	7, 8
Bruker	9
Aminoacids.com	10
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Technical Presentations – Thursday, May 9, 2024

8:45 **Introduction**
Garden City Ballroom

9:00 **Keynote Address: Dr. Katelynn Perrault Uptmor**
Department of Chemistry, College of William & Mary
"*Multidimensional Chromatography Analyses as an Investigative Tool*"

10:00 **Morning Break –** Exhibits & Poster Session – Carriage Hall & Atrium

Poster #1	The wikiChrom Project – Principles, Results, and Knowledge Grace LaTourelle and Ella Sontowski
Poster #2	Preliminary Characterization of a Feed Injection Approach for Liquid Chromatography Sarah Carr
Poster #3	Enhancing High Throughput Preparative HPLC: Offline Regeneration of Identical Superficially Porous Particle Preparative Columns to extend Column Lifetime, Minimize Sample Carryover and Increase Throughput Lori Sandford
Poster #4	The Study of Visual Chromophore Logistics and Supply: Ocular and Systemic Vitamin A Metabolite Detection and Quantification through Normal Phase High Performance Liquid Chromatography Matthias Leung
Poster #5	Ultratrace Analysis of Neonicotinoids in Surface Waters using Ice Concentration Linked with Extractive Stirrer and High-Performance Liquid Chromatography-Tandem Mass Spectrometry Mawuli MacDonald

10:30 – 12:00 **Morning Session**

Time	**Captain's Room (Main Level)**
	Sample Preparation and Environmental Analysis
10:30 – 11:00	Development of Dry-Herb Vaporizer-Assisted Solid-Phase Microextraction for the Analysis of Volatile Organic Compounds Douglas Raynie
11:00 – 11:20	The Occurrence and Removal of Antibiotics in Wastewater Treatment During the COVID-19 Pandemic Zihang Wang
11:20 – 11:40	Enhancing the Interactions of Ion-Tagged Oligonucleotides and Magnetic Ionic Liquid Supports for the Sequence-Specific Extraction of DNA Seong-Soo Lee
11:40 – 12:00	Advancing Plant Biomolecular Analysis: DNA Extraction from Diverse Plant Taxa Featuring Ionic Liquids and Magnetic Ionic Liquids Shashini De Silva

12:00 – 1:30 **Lunch** – Garden City Ballroom

12:00 – 2:00 **Exhibit Hall Open** – Carriage Hall

1:30 – 4:00 **Afternoon Sessions**

Time	Captain's Room (Main Level)
	Stationary Phases and Mobile Phases
1:30 – 2:00	Investigating Novel GC Column Chemistries for Improved Performance, Sensitivity and Data Quality and Accuracy Daron Decker
2:00 – 2:20	An Investigation into the Potential of Propylene Carbonate as a Green(er) Replacement for Acetonitrile in Reversed-Phase Liquid Chromatography Zachary Kruger
2:20 – 2:40	Big(ger) Chromatographic Data Enables Prediction of Isomer Selectivity in Liquid Chromatography Dwight Stoll

2:40 – 3:00 **Break**

Time	Captain's Room (Main Level)	Tack A/B (Lower Level)
	Novel Methodologies	**Pharmaceutical Analysis**
3:00 – 3:20	Smartphone as a Luminescence Detector for High-performance Liquid Chromatography Danial Shamsaei	Automated flow-based analysis for biopharmaceuticals evaluation Diana Cunha
3:20 – 3:40	An Aliquot Push-Pull Interface for Flexible Sampling of the First Dimension Separation in Two-Dimensional Liquid Chromatography Ryan Schimek	Development of Multiple Heartcutting Two-Dimensional Liquid Chromatography with Ion-Pairing Reversed-Phase and Hydrophilic Interaction Chromatography for Characterization of Impurities in Therapeutic Oligonucleotides Daniel Meston
3:40 – 4:00	Optimization of High-throughput Discovery-based Proteomics Workflows LeeAnn Higgins	Triple Drug Assay for Simultaneous Measurement of Levetriacetam, Ceftriaxone, and Atorvastatin Concentrations in Rat Plasma and Brain Samples Usha Mishra
4:00	**End of Technical Program**	

3:30 – 4:30 **Exhibit Hall Open** – Carriage Hall

4:10 **Door Prize Drawing** – Carriage Hall

Keynote

Multidimensional Chromatography Analyses as an Investigative Tool

Katelynn Perrault Uptmor

College of William & Mary, Williamsburg, VA, USA

Abstract

While we encounter numerous odors throughout the course of the day, the average person is largely unaware of the considerable analytical potential of such molecules. Odors are comprised of a complex array of volatile organic compounds (VOCs), typically consisting of hundreds of analytes across a wide range of chemical classes. As a result, odors have vast probative value due to the complex chemical signature that can be used to differentiate samples from one another. There are numerous chemical applications that rely on the detection and differentiation of odors. This presentation will highlight the link between scent and odor, while describing the current state-of-the-art in VOC collection and analysis using multidimensional chromatography. In particular, the technique known as comprehensive two-dimensional gas chromatography (GC×GC) will be described, as well as some of our uses of this technique in forensic and life science applications.

1 (Poster #1)

The wikiChrom Project – Principles, Results, and Knowledge

Ella Sontowski, Grace LaTourelle, Zachary Kruger, Trevor Kempen, Tina Dahlseid, Bob Pirok, and Dwight Stoll

Gustavus Adolphus College

Abstract

Efforts to model and simulate various aspects of liquid chromatography (LC) separations (e.g., retention, selectivity, peak capacity, injection breakthrough) depend on experimental retention measurements to use as the basis for the models and simulations. Often these modeling and simulation efforts are limited by datasets that are too small because of the cost (time and money) associated with making the measurements. Other groups have demonstrated improvements in throughput of LC separations by focusing on "overhead" associated with the instrument itself – for example, between-analysis software processing time, and autosampler motions. In our wikiChrom Project we are focused on improving the throughput of retention measurements such that datasets of hundreds of thousands of measurements can be made on a practical timescale (e.g., a few years). In this poster we will describe our approach, which is focused on the use of low volume columns operated at high flow rates, as well as instrumentation and informatics infrastructure that make this possible. We use ratios of retention factors (i.e., selectivities) to translate retention measurements between columns of different dimensions, so that measurements made using small columns can be used to make predictions for separations that involve conventional columns. With about 50,000 measurements in-hand, we have made several important observations that both help to set expectations regarding the characteristics of the data, and guide refinement of the platform as we continue making measurements into the future: 1) measured selectivities (i.e., ratios of retention factors) are much more stable over time than retention factors ; 2) we find that short-term retention measurement precision is good (most RSDs less than 1%), and intermediate-term reproducibility is good (less than 1% drift over six months for many compounds); and 3) lot-to-lot variability in the selectivity of columns and drift in selectivity over time are going to be serious challenges that we will have to monitor very closely going forward. This approach significantly increases the rate at which high quality retention data can be collected to thousands of measurements per instrument per day, which in turn will likely have a profound impact on the quality of models and simulations that can be developed for many aspects of LC separations. Finally, all of the data acquired as part of this project will be made freely available through a website shared with the poster.

2 (Poster #2)

Preliminary Characterization of a Feed Injection Approach for Liquid Chromatography

Sarah Carr, Zachary Kruger, and Dwight Stoll

Gustavus Adolphus College

Abstract

Many applications of liquid chromatography involve samples with diluents very different in composition from the mobile phase used for the LC separation. This mismatch between diluent and mobile phase can negatively affect the quality of the separation, resulting in distorted and wider-than-expected peaks. Recently, commercial instrumentation was introduced that enables “feed injection”, which is one way to address the mismatch problem. In contrast to conventional injections in LC where the entire volume of the injected sample is introduced into the mobile phase in one plug, in feed injection a portion of the sample is slowly “fed” into the mobile phase such that it mixes with the mobile phase before reaching the column. In this way, the mobile phase itself can be used as a diluent to reduce the degree of mismatch, thereby reducing the likelihood of negative affects of the sample diluent on the separation.

In this presentation we will share preliminary results of experiments aimed at a basic characterization of the instrument hardware used for feed injection. We are carefully studying the sample ejection profile observed during the feed injection process. Observations made during these experiments will be used to optimize future methods developed using the feed injection approach.

3 (Poster #3)

Enhancing High Throughput Preparative HPLC: Offline Regeneration of Identical Superficially Porous Particle Preparative Columns to extend Column Lifetime, Minimize Sample Carryover and Increase Throughput

Lori Sandford, Steve Rosa

Agilent Technologies

Abstract

High throughput sample purification and analysis are important tasks in many industries. With the increasing number of samples synthesized– HPLC purification should not be the bottleneck in drug discovery and development. The throughput on an HPLC system can be increased by optimizing the chromatographic method and by optimizing the instrument hardware. In this poster we show how the throughput can be enhanced even further by using two identical Poroshell Preparative HPLC columns in the system and operating them alternately optimizing the 1290 Infinity II Preparative LC System for Off-Line Column Regeneration. The greatest benefit of the offline column cleanup and equilibration is the increase in sample throughput, but added benefits include extended column lifetime and a reduction in sample carryover.

4 (Poster #4)

The Study of Visual Chromophore Logistics and Supply: Ocular and Systemic Vitamin A Metabolite Detection and Quantification through Normal Phase High Performance Liquid Chromatography

Matthias Leung, Rakesh Radhakrishnan, Glenn Lobo

Department of Ophthalmology and Visual Neurosciences, University of Minnesota

Abstract

In all mammalian vision, rhodopsin is the G protein-coupled receptor (GPCR) responsible for initiation of the phototransduction cascade. Within photoreceptors, rhodopsin is bound to its chromophore 11-cis retinal and is activated through the light sensitive isomerization of 11-cis retinal to all-trans retinal, which activates the transducin G Protein, resulting in the phototransduction cascade. While phototransduction is well understood, the processes that are involved in the supply of 11-cis retinal are not fully understood. 11-cis retinal is a molecule within a family of molecules called vitamin A and originates from dietary sources such as β-carotene. Once vitamin A precursors are absorbed into the intestine, it is stored in the liver as retinyl esters, and released into the bloodstream as all-trans retinol bound to retinol binding protein 4. This circulatory retinol will be absorbed by systemic organs, such as the lungs, kidney, and eye. Hence, a method for the quantification of the various metabolites of vitamin A in the eye and systemic organs is critical to the study proper rhodopsin GPCR function. In this method, we present an extraction and analytical method for vitamin A analysis in murine tissue. Through normal phase analysis, all relevant isomers of retinal, retinol, and retinyl esters can be detected simultaneously through a single analysis, which allows for efficient use of experimental samples and increases internal reliability across different vitamin A metabolites within the same sample. With this comprehensive method, further investigators will be able to better assess systemic vitamin A supply in rhodopsin GPCR function.

5 (Poster #5)

Ultratrace Analysis of Neonicotinoids in Surface Waters using Ice Concentration Linked with Extractive Stirrer and High-Performance Liquid Chromatography-Tandem Mass Spectrometry

Mawuli MacDonald, Kyle Burch, Brian Logue

Department of Chemistry, Biochemistry and Physics, South Dakota State University

Abstract

Neonicotinoids (neonics) are the most widely used neurotoxic insecticides in the world, accounting for almost one-third of all pesticides used. As a result of their widespread use, high water solubility and moderate bio-persistence, they are ubiquitous in surface waters and have been shown to be harmful to aquatic life. Due to the intermediately hydrophobic nature of neonicotinoids, extraction into typical sorbent phases is usually difficult during sample preparation. Therefore, a method for the ultratrace analysis of neonicotinoids in surface waters using Ice Concentration Linked with Extractive Stirrer (ICECLES) and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was developed to address this issue. While the method is still under development, it is currently able to detect five major neonicotinoids (imidacloprid, clothianidin, thiamethoxam, thiacloprid, and acetamiprid) at chronic limits (10 ng/L) for aquatic life set by the EPA in deionized water. Total sample volume required for extraction is 15 mL. The extraction process is automated and requires less than 300 µL of organic solvents. Finally, the method described above allows for re-use of consumables, thereby making it cost effective. Validation and testing on real surface water samples is about to begin. In summary, this method shows promise to be a simple, sensitive, and environmentally friendly way to analyze neonicotinoids in surface waters.

6

Development of Dry-Herb Vaporizer-Assisted Solid-Phase Microextraction for the Analysis of Volatile Organic Compounds

Douglas Raynie, and AKM Ahsan Ahmed

South Dakota State University

Abstract

A dry-herb vaporizer (i.e., vaping pen) was used as a sample heating device for direct desorption of volatile analytes from solid samples into the headspace of the device. The extraction was performed by sorbing these gas-phase analytes in the headspace of the vaporizer onto a solid-phase microextraction (SPME) fiber followed by desorption in the injection port for gas chromatography-mass spectrometry (GC-MS) characterization. Horseradish, cinnamon, and gasoline-spiked soil samples were analyzed. The samples were also analyzed by conventional headspace SPME-GC-MS. The results from both methods were compared. The methods produce comparable results in terms of extracted compounds and relative area percentages, however, the dry-herb vaporizer had improved sensitivity. While further development is needed, this approach promises great potential for inexpensive and sensitive field-portable analysis while offering a range of desorption temperatures not commonly found in typical methods such as headspace sampling, thermal desorption, or pyrolysis.

7

The Occurrence and Removal of Antibiotics in Wastewater Treatment During the COVID-19 Pandemic

Zihang Wang[1], William Arnold[1], and Huan He[2]

[1]University of Minnesota
[2]Tongji University, Shanghai, China

Abstract

Due to the COVID-19 pandemic, various antibiotics, such as azithromycin, were applied at a greater frequency to treat infected patients, supposedly increasing the load to wastewater treatment plants (WWTPs) and water systems, rising significant concerns to the environment and public health. From August 2020 to July 2022, monthly influent and effluent samples were collected from four WWTPs with treatment capacities of 20-200 million gallons/day, serving a total population of ~2500000 people, to investigate the occurrence, concentration, and removal efficiency of antibiotic. Profiles of the 26 studied antibiotics in each class were dominated by the compounds used by humans instead of those solely for animals, i.e., sulfamethoxazole for sulfonamides, azithromycin for macrolides, doxycycline for tetracyclines, ciprofloxacin for fluoroquinolones, and trimethoprim for the other compounds. WWTPs effectively removed certain antibiotics (on average 47%–88% for sulfamethoxazole, and 53%-80% for ciprofloxacin), while negative removals were observed in other cases (possibly due to adsorption of antibiotics to particles/flocs in the influent samples leading to lower recoveries). Concentrations of the prevalent compounds were 0 to ~4000 ng/L in the effluent samples. The high concentrations of azithromycin (AZRM) were observed during the initial stages of the COVID-19 pandemic, which spiked approximately four weeks after a spike of weekly vial loads in WWTPs. The concentration of AZRM detected in influents positively correlated with weekly reported hospitalizations ($\rho = 0.786$; $p = 0.05$).

8

Enhancing the Interactions of Ion-Tagged Oligonucleotides and Magnetic Ionic Liquid Supports for the Sequence-Specific Extraction of DNA

Seong-Soo Lee, Derek R. Eitzmann, and Jared L. Anderson

Iowa State University

Abstract

Circulating cell-free DNA (ccfDNA) can be a promising biomarker for diagnosis and prognosis of cancer because the level of ccfDNA in blood samples of cancer patients (180 $ng \cdot mL^{-1}$) is significantly higher than the level in the samples of healthy individuals (30 $ng \cdot mL^{-1}$). However, there is a possible drawback that the amplification of small amounts of mutant ccfDNA may not be the primary component in a sample from an early-stage cancer patient because of low abundance and single nucleotide polymorphism (SNP)s originating from mutant ccfDNA in the sample. Therefore, the challenge of isolating and enriching specific target DNA from biological matrix must be resolved to secure high accuracy and precision of diagnosis with ccfDNA. As novel sequence-specific isolation and purification techniques, the methodology using ion-tagged oligonucleotides (ITOs) as probes and magnetic ionic liquids (MILs) as liquid supports is a promising method that resolves the drawbacks of streptavidin-coated magnetic beads, including its high cost and vulnerability to aggregation and sedimentation. In this study, diverse functional groups of ITOs were prepared to enhance hydrophobic or fluorophilic interactions between ITOs and MIL to improve the efficiency of sequence-specific DNA extraction. ITOs were synthesized via thiol-ene and thiol-yne click chemistry, purified and obtained by polyacrylamide gel electrophoresis. The prepared ITOs were characterized by LC-TOFMS using Zorbax Extend-C18 column with the mobile phases of triethylammonium acetate and acetonitrile. The loading efficiency of ITO on MIL was indirectly evaluated by measuring the residue of ITO after the extraction by HPLC with the anion-exchange column. The ITO probe substituted with a branched alkyl group (C16) showed the highest loading efficiency in all prepared ITOs. The sequence-specific isolation and purification of a 215 bp target sequence from spiked aqueous solution by ITO-MIL method is successfully validated by real-time quantitative polymerase chain reaction (qPCR) amplification.

9

Advancing Plant Biomolecular Analysis: DNA Extraction from Diverse Plant Taxa Featuring Ionic Liquids and Magnetic Ionic Liquids

Shashini De Silva,[1] Cecilia Cagliero,[2] Morgan R. Gostel,[3] Gabriel Johnson[4] and Jared L. Anderson[1]

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Abstract

The demand for rapid and reliable tools for plant biomolecular analyses is increasing. DNA isolation remains the limiting step in plant nucleic acid-based applications especially due to the complexity of plant tissues across different species. Traditional plant DNA extraction methods typically require extensive sample preparation, substantial quantities of sample and chemicals, high temperatures, and multiple sample transfer steps which pose challenges in high throughput applications. This study introduces a breakthrough in plant DNA isolation with a simple, miniaturized approach employing ionic liquids and magnetic ionic liquids to extract DNA from milligram fragments of plant tissue across a broad range of plant taxa. This approach was notably successful in extracting DNA from a century old herbarium sample. The quality of the extracted DNA was validated through successful amplification by quantitative polymerase chain reaction (qPCR). PCR products were analyzed by gel electrophoresis followed by Sanger sequencing for a representative dicot and monocot species. The extracted DNA can be stored at room temperature for an extended time period up to 3 weeks prior to analysis. This innovative approach reduces the need for large amounts of sample and solvents and has tremendous potential for various applications in plant biology that require DNA, including barcoding methods for agriculture, conservation, ecology, evolution, and forensics.

10

Investigating Novel GC Column Chemistries for Improved Performance, Sensitivity and Data Quality and Accuracy

Daron Decker, Vanessa Abercrombie, Ashlee Gerardi, Dale Walker

Agilent Technologies

Abstract

Developments in mass spectrometry sensitivities continue to push the limits of detection in gas chromatography. As source technologies advance, column technology must advance as well. Increased detection of analytes also results in increased detection of background including column bleed. Therefore, GC column technology that lowers bleed can enhance sensitivity and data accuracy. In this presentation, we will examine how column attributes, like bleed, thermal stability and inertness can translate into practical improvements in data quality and accuracy, instrument sensitivity and performance and overall productivity for the lab.

11

An Investigation into the Potential of Propylene Carbonate as a Green(er) Replacement for Acetonitrile in Reversed-Phase Liquid Chromatography

Zachary Kruger, Daniel Meston, Ella Sontowski, Grace LaTourelle, and Dwight Stoll

Gustavus Adolphus College

Abstract

Replacement of acetonitrile (ACN) as an organic modifier in reversed-phase high performance liquid chromatography (RP-HPLC) experiments is a growing area of interest in chromatography. RP-HPLC is the most commonly used mode of liquid chromatography, and typically employs ACN as the organic modifier due to its low UV cutoff (~190 nm), low viscosity, and unique chromatographic selectivity. With environmental awareness emerging as an important topic in analytical science, numerous solvent ranking systems have seen ACN fall behind the likes of other possible organic mobile phase modifiers such as methanol, ethanol, and isopropanol. While the chromatographic use of methanol as an organic solvent is not uncommon, other greener alternatives such as ethanol and propylene carbonate are not as well documented. Using a high-throughput approach, retention data for 58 compounds was collected using either ACN or a propylene carbonate/methanol mixture as the organic mobile phase component. A modified Neue-Kuss model of retention was used to determine analyte specific retention parameters and assess resolution and selectivity differences between ACN and propylene carbonate/methanol. In this presentation we will discuss trends in selectivity related to specific functional groups, leading to unique separation results between propylene carbonate/methanol and ACN. Most notably, while certain analytes are separable in ACN but not propylene carbonate, we also observe the reverse case, providing insight into the unique possibilities of using propylene carbonate in RP-HPLC separations.

12

Big(ger) Chromatographic Improves Prediction of Isomer Selectivity in Liquid Chromatography

Dwight Stoll[1], Trevor Kempen[1], Tina Dahlseid[1], Zachary Kruger[1], Bob Pirok[1], Jonathan G. Shackman[2], Yiyang Zhou[2], Qinggang Wang[2] and Sarah C. Rutan[3]

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[3]Department of Chemistry, Box 842006, Virginia Commonwealth University, Richmond, VA 23284-2006

Abstract

Despite the popularity of reversed phase liquid chromatographic separations to address a wide range of analytical problems, there has yet to be a comprehensive model developed for the accurate prediction of separation selectivity over a broad range of potential analytes. One part of the solution to the lack of a sufficient model for a description of chromatographic selectivity is to have available large sets of retention data for a broad range of solutes on many different types of stationary phases and mobile phases. We have recently developed methods to enable high-throughput measurement of retention, which has made available to us a very large dataset of retention data. In this work, we focus on the analysis of selectivity data for a set of 75 compounds on 13 diverse reversed phase stationary phases. We have used a strategy analogous to the hydrophobic subtraction model, in use now for over 20 years [1], to develop the following model

$$\ln\alpha = \ln\left(\frac{k_x}{k_{EB}}\right) = hH + kC + aB + bA + dD + eE + sS$$

Here, α is the selectivity of solute *x*, compared to ethyl benzene (EB), and *h*, *k*, *a*, *b*, *d, e and s* are solute parameters for hydrophobicity, ionic interactions, hydrogen bond acidity, hydrogen bond basicity, solute dipolarity, solute polarizability and steric effects, respectively, and *H*, *C*, *B*, *A*, *D*, *E* and *S* are the complementary stationary phase parameters. This is a data-driven model; thus, the predictions are as accurate as a 7-component principal components model. The range of molecular weights encompassed by the new dataset is doubled relative to the solutes used for the original hydrophobic subtraction model, ensuring that the new model should have wider applicability.

Based on the above the model, we have attempted to rationalize the retention of the selectivity of positional isomers, isomers with shape variations, and closely related compounds of pharmaceutical significance. In some cases, we can rationalize the observed differences in selectivity for positional and geometric isomers based on these model parameters. The results of the present study demonstrate the power of large data sets to help in understanding the drivers in reversed phase selectivity.

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, The hydrophobic-subtraction model of reversed-phase column selectivity, J. Chromatogr. A 1060 (2004) 77–116. https://doi.org/10.1016/j.chroma.2004.08.121.

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Smartphone as a Luminescence Detector for High-Performance Liquid Chromatography

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Abstract

Luminescence-based detection, specifically fluorescence and chemiluminescence, has become widely employed in high-performance liquid chromatography (HPLC) due to its high specificity and sensitivity. However, the traditional implementation of these techniques is often limited by their requirements of bulky and expensive instrumentation. Recent advances in electronics and technology have facilitated the use of smartphones as portable tools for recording, analyzing, and monitoring, which has prompted significant interest in developing low-cost, user-friendly, and portable detection methods.

This presentation will discuss a miniaturized, portable, user-friendly, and low-cost smartphonebased HPLC luminescence detector for the detection of six different coumarin dyes using a smartphonebased fluorescence detector, and carbamazepine using a smartphone-based chemiluminescence detector. The smartphone is able to capture, record, and analyze emitted luminescence light, providing chromatograms in three detection channels by plotting the red, green, and blue (RGB) color intensities versus time. The fluorescence detection setup includes a microfluidic device acting as a flow cell, a UV-LED as a light source to excite fluorescent molecules, 3D-printed housing, and fiber optic cables to transfer the fluorescent light to the smartphone camera. The chemiluminescence detector incorporates a fully 3D-printed flow cell and housing designed to isolate chemiluminescence emission from surrounding light and accommodate all necessary components, along with mixers to ensure appropriate mixing of the analyte and reagents. The presentation will detail the optimization of key experimental parameters, such as the design and fabrication of the flow cell, the design of the mixer, and smartphone parameters, as well as discuss their effects on peak broadening and detector response.

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Automated flow-based analysis for biopharmaceuticals evaluation

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Abstract

Analytical chemists face diverse challenges in pharmaceutical development, particularly with the emergence of biopharmaceutical therapies. For instance, more than 197 biopharmaceuticals have been approved by EMA and by FDA between January 2018 and June 2022. Monoclonal antibodies (mAbs) are the largest group, representing 53.5% of the biopharmaceuticals approved in that timeframe [1]. These innovative medicines have prompted a need for advanced analytical methods capable of characterizing the complex properties of biological structures ensuring the quality of these medicines [2], namely, to assess specific glycosylation attributes [3]. The automation of molecular recognition strategies presents several advantages within the sphere of analytical chemistry. Remarkably, it mitigates human error, enables high-throughput analysis throughput, and allows the optimization and standardization of experimental protocols. Furthermore, automated systems can afford the integration of multiple analytical techniques, such as chromatographic methods, thereby enabling multidimensional analyses and enhancing the overall sensitivity and selectivity of the methodologies employed [4]. Automation has significantly advanced (bio)chemical analysis, particularly in the realm of solid-phase extraction (SPE), offering precise control over reaction time and facilitating repeatable contact between analytes and recognition elements that are immobilized or present in solid supports. This enhances analytical figures of merit, including accuracy, precision, and sample throughput, while aligning with the principles of Green Analytical Chemistry (GAC) [4]. In this context, the present communication aims to highlight the importance and contribution of flow-based methods to support pharmaceutical development, particularly the role of sample treatment of biopharmaceuticals. Using the micro-bead injection spectroscopy (μ-BIS), it becomes possible the consistent packing of microaffinity columns between two optical fibers. While the biopharmaceuticals retention occurs, unbound molecules are carried away. Given the lab-on-valve (LOV) μ-BIS conditions, the immobilization of biopharmaceuticals can be directly assessed on the column. Flow programming enables precise control of solution handling and the time of interaction between the biopharmaceuticals and the solid support, ensuring consistent immobilization under non-equilibrium conditions [5]. Thus, was possible the evaluation of mAbs capture using immobilized Concanavalin A in Sepharose beads through automated BI-LOV, with the real-tome monitoring and quantification performed using in situ spectrophotometry.

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References

[1] Walsh G. Walsh E, Nature Biotechnol. 40 (2022) 1722.

[2] L. Zhang, S. Luo, B. Zhang, mAbs, 8 (2016) 205.

[3] V.E. Abrantes-Coutinho, A. Santos, R. Moura, F. Junior, L. Mascaro, S. Morais, T. Oliveira, Colloids and Surfaces B-Biointerfaces, 208 (2021) 14.

[4] Gutzman, Y., A.D. Carroll, and J. Ruzicka, Analyst, 131 (2006) 809.

[5] I.I. Ramos, S.S. Marques, L.M. Magalhães, L. Barreiros, S. Reis, J.L.F.C. Lima, M.A. Segundo, Talanta, 204 (2019) 547

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An Aliquot Push-Pull Interface for Coupling the First and Second Dimension Separations in Two-dimensional Liquid Chromatography

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Abstract

Two-dimensional liquid chromatography (2D-LC) separations are increasing in popularity, both for the analysis of relatively simple, but hard-to-separate mixtures (e.g., mixtures of achiral and chiral compounds), and the analysis of highly complex mixtures such as those encountered in the biological samples and natural products. Despite tremendous advances in many aspects of the instrument hardware and software needed for routine use of 2D-LC, there are some aspects of the technology that have hardly changed since the first online 2D separations were demonstrated several decades ago. In this presentation we will describe a novel, alternative approach to interfacing the first and second dimensions of separation that addresses some of the shortcomings of existing technology. The approach – which we descriptively refer to as a "push-pull" interface – lets go of the conventional fixed loop feature of existing interfaces, and uses a precisely controlled, high pressure syringe to first "pull" in an aliquot of first dimension effluent that we desire to transfer to a second dimension for further separation. Then, it is "pushed" into the mobile phase stream of the second dimension and carried to the second dimension column. There are several advantages of this approach over existing ones, including software control of both the aliquot volume, and the rate at which the aliquot is fed into the second dimension mobile phase stream. In addition to an explanation of the modulation cycle, we will share results of preliminary work to illustrate the basic features of the interface/approach and show application examples that highlight the advantages of the push-pull interface over existing interfaces for 2D-LC.

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Development of Multiple Heartcutting Two-Dimensional Liquid Chromatography with Ion-Pairing Reversed-Phase and Hydrophilic Interaction Chromatography for Characterization of Impurities in Therapeutic Oligonucleotides

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Abstract

High resolution methods for characterization of therapeutic oligonucleotides (ONs) are becoming increasingly important as the number of candidate therapeutics in this class in the drug development pipeline continues to increase. It is becoming clear that conventional one-dimensional separation methodologies are limited in their ability to fully resolve all impurities of interest for synthetic ONs. Two-dimensional liquid chromatography (2D-LC) holds great promise for addressing these limitations wherein, unresolved mixtures sampled from the first dimension (^{1}D) separation can be resolved by the second dimension (^{2}D) separation.

In this presentation we will focus on three main developments. First, we will discuss the complementarity of selectivities for ion-pairing reversed-phase (IPRP) and HILIC separations of several impurities of a therapeutic ON, and the utility of this complementarity in development of 2D-LC methods. Second, we will demonstrate the effectiveness of our systematic method development workflow when developing a 2D-LC method involving a HILIC separation in the second dimension. Third, we will discuss results that show – for the first time to the best of our knowledge – a situation where dilution of the ^{1}D column effluent with weak solvent to mitigate mobile phase mismatch problems does not yield the best ^{2}D separations. Instead, we observe that the diluent has to be optimized to yield the best results.

Finally, we will use 2D separations of impurities present following ON synthesis to illustrate that the 2D method both is sufficiently sensitive to quantify impurities down to the 0.3% level, and enables identification using mass spectrometric detection.

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Optimization of High-throughput Discovery-based Proteomics Workflows

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Abstract

Complex peptide mixtures are typically analyzed by liquid chromatography-mass spectrometry (LC-MS) in discovery-based proteomics experiments. A common goal for a proteomics experiment is detection and or quantification of the maximum number of proteins/peptides in a set of samples. In the past few decades, mass spectrometry-based proteomics research has included multi-dimensional separation techniques such as 2D gel separation of proteins and 2D LC separation of peptides, in addition to ion mobility applications for peptide separation in the gas phase. Extra dimensions of separation typically require extra processing time per sample unless custom instrumentation is available for faster throughput. Long sample processing times are unideal for research or clinical studies with large cohorts and as a result, efforts have been made to improve throughput with computational advances in the proteomics field. The new advances in machine learning applications for prediction of peptide fragmentation events and advancements in peptide library search techniques have resulted in a shift in the traditional paradigm of data acquisition for complex peptide mixtures. Historically, data acquisition methods and the corresponding software for analysis were designed for single peptide tandem MS events referred to as Data Dependent Acquisition (DDA). Newer mass spectrometry and data analysis methods are catered to mixtures of co-eluting or nearly co-eluting peptides, eliminating the need for extensive peptide separation. We introduce the Data Independent Acquisition (DIA) mass spectrometry methods used for analysis of complex mixtures in 'short' run times and provide summaries of results from applications uniquely designed to handle complex peptide tandem MS data.

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Triple Drug Assay for Simultaneous Measurement of Levetriacetam, Ceftriaxone, and Atorvastatin Concentrations in Rat Plasma and Brain Samples

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Abstract

Simultaneously analysis of drugs in rat plasma and brain tissue samples with different ionization and lipophilic properties is very challenging; however, an LC-MS/MS method was developed and validated for the simultaneous analysis of Atorvastatin, Ceftriaxone, and Levetiracetam in rat plasma and brain tissue. The analytes and internal standards were detected using the MRM (Multiple Reaction Monitoring} mode on a TSQ Quantum Max Access mass spectrometer with positive electrospray ionization. The three drugs were separated using the Agilent InfinityLab, Poroshell 120 EC8 (3.0 mm x 150 mm) column and mobile consisting of 0.1% formic acid and 0.1% formic acid in acetonitrile as the mobile phase. Sample preparation involved acidification of plasma and protein precipitation using methanol and acetonitrile. Concentration ranges from 10 to 500 ng/mL atorvastatin, and 2-100 µg/mL for Levetiracetam and Ceftriaxone were linear. The accuracy and precision for all three drugs ranged within an acceptable range. The method successfully measured three drugs in rat plasma and brain samples.