PROGRAM AND ABSTRACTS

Connecticut Separation Science Council
Presents

9th Annual Csaba Horváth Medal Award Symposium

Honoring
Professor Georges Guiochon

April 28 – 29, 2009

Harford Convention Center, Hartford CT

Dear Participants,

On behalf of the Officers, Board of Directors, and the Organizing Committee of the Connecticut Separation Science Council (CSSC) we welcome you to the 9th Annual Csaba Horváth Medal Award Symposium.

Csaba Horváth was one of the foremost figures in the field of separation science. The American Chemical Society includes Professor Csaba Horváth among great scientists such as Crick and Watson, Linus Pauling, Pierre and Marie Curie, and Ernest Rutherford -- individuals who have contributed most significantly to the development of chemistry in the 20th century. Along with J. Calvin Giddings and J.F.K. Huber, Csaba Horváth developed the concept of the first HPLC instruments. His seminal works form the basis of modern separation science.

At the 1999 CSSC Annual Board Meeting held at Yale University, Professor Horváth suggested the establishment of a CSSC Medal. The CSSC bylaws require that the medal "… be awarded to a recognized person in the field of separation science whose work has exemplified and will continue to provide for the advancement of separation science in the future." For his contributions, Csaba was awarded the first medal. Subsequent recipients of the medal were Dr. Leslie Ettre, Dr. William S. Hancock, Dr. Harold McNair, Dr. Phyllis Brown, Dr. Lloyd Snyder, Dr. Eli Grushka, Dr. Pier Giorgio Righetti, and Dr. Barry Karger.

This year, we are honored to award the medal to Dr. Georges A. Guiochon. Dr. Guiochon received his Ph.D. in chemistry from the University of Paris in 1958. He was a Professor of Chemistry at Ecole Polytechnique (1958-1985) and at the University Pierre et Marie Curie of Paris (1968-1984), then at Georgetown University in Washington, D.C. (1984-1987). He was appointed a Distinguished Professor at the University of Tennessee (Department of Chemistry) and a Senior Scientist at the Oak Ridge National Laboratory (Division of Chemical Sciences) in June 1987.

Dr. Guiochon's fundamental contributions to separation sciences in general and chromatography in particular, place him in the forefront of the field together with the modern founders of chromatography -- A. J. P. Martin, R. L. M. Synge, J. C. Giddings and C. Horváth. His research includes all aspects of gas and liquid chromatography, theory, instrumentation and applications, and the problems of physical chemistry related to chromatography. These include solution and adsorption thermodynamics, mass and energy transfers, detector principles, and the consolidation of beds of fine particles. His current work is in the theory of nonlinear chromatography, the development of separation processes based on chromatography (including SMB), and the preparation of efficient columns. He has more than 950 scientific publications. His most recent books are Quantitative Gas Chromatography, written in cooperation with C. Guillemin (1988), Fundamentals of Preparative and Nonlinear Chromatography, written in cooperation with S. G. Shirazi and A. M. Katti (1994, 2006), and Modeling for Preparative Chromatography, written in cooperation with B. Lin (2003).

He is a member of the editorial boards of Chromatographia, the Journal of Chromatography A, the Journal of Liquid Chromatography, and the Journal of Chromatographic Sciences. He was the first foreign member of the Editorial Advisory Board of Analytical Chemistry (1979-82) and was the Associate Editor of the Journal of Separation Sciences from 1985-93.

His achievements include numerous national and international awards. We are proud to add CSSC's 2009 Csaba Horváth Medal Award to this list.

Finally we wish to acknowledge the contributions of Ms. Cristina Manolescu, Dr. Yuwen Wang, Dr. Shengli Ma and Sherry Shen of Boehringer Ingelheim Pharmaceuticals, Inc., Ms. Kim Johnson of Bristol Myers Squibb, Professor James D. Stuart of the University of Connecticut, and Dr. George Reed and Dr. Gang Xue of Pfizer, for their contributions to this event.

Nelu Grinberg, Ph.D.
President CSSC
Distinguished Scientist
Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT

John McCaffrey, Ph.D.
Vice President CSSC
Associate Senior Director
Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT

WINNERS OF THE CSABA HORVÁTH MEDAL

2000 - DR. CSABA HORVÁTH

2001 - DR. LESLIE ETTRE

2002 - DR. WILLIAM S. HANCOCK

2003 - DR. HAROLD MCNAIR

2004 - DR. PHYLLIS BROWN

2005 - DR. LLOYD SNYDER

2007 - DR. ELI GRUSHKA

2008 – DR. BARRY KARGER

2008 – DR. PIER GIORGIO RIGHETTI

2009 – DR. GEORGES GUIOCHON

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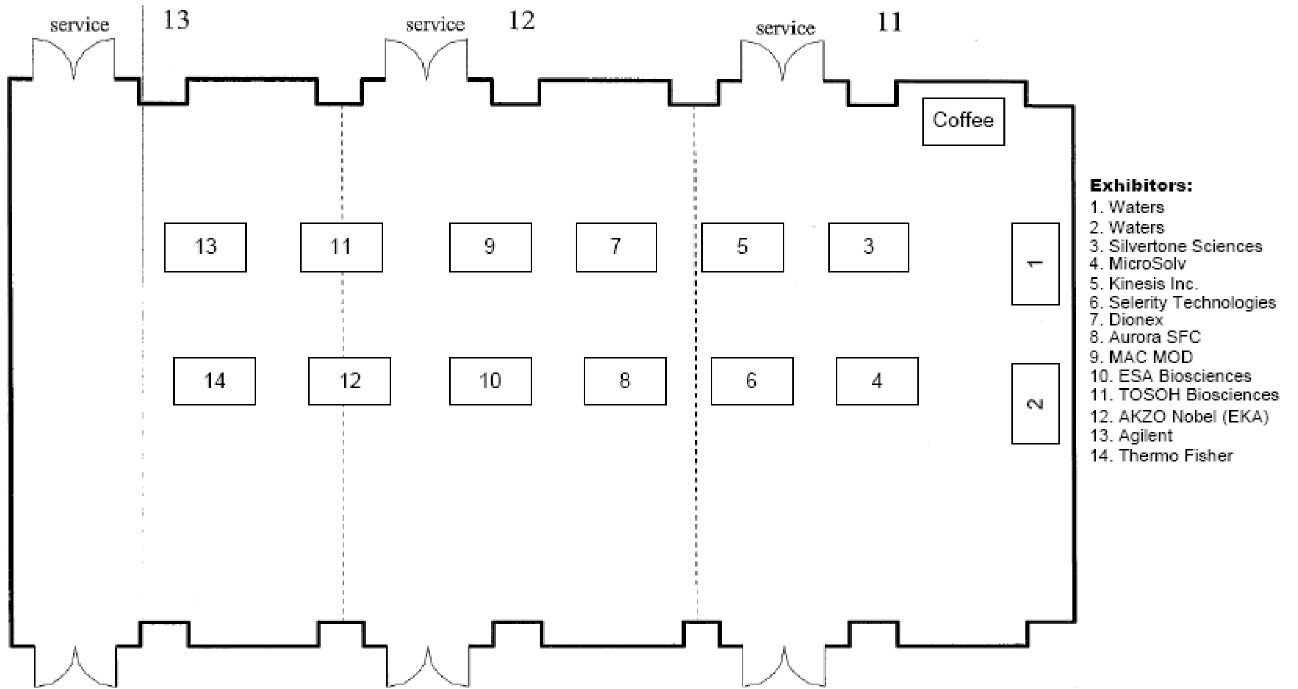

9th Csaba Horvath Medal Award Symposium - Vendors' Exhibit Floor Plan

FINAL PROGRAM FOR 9TH CSABA HORVÁTH MEDAL AWARD SYMPOSIUM

TUESDAY APRIL 28TH 2009

7:30 – 8:20 – Registration and Breakfast

8:20 – 8:30 - 9th Annual Csaba Horváth Symposium kick off

Section 1: Separation Techniques

Chair: Attila Felinger, University of Pecs, Pecs, Hungary

8:30 – 9:00 O#1 Plenary Lecture
Some Insights on Retention and Selectivity for Hydrophilic Interaction Chromatography
Richard Thompson and M. Yang - Novartis Pharmaceuticals Corporation, East Hanover, NJ

9:00 – 9:20 O#2 Pushing the envelope on separation speed: "knowing when to push it and when to back off"
Mark J. Hayward - Lundbeck Research USA - Paramus, NJ 07652

9:20 – 9:40 O#3 Evolution of Capillary Electrophoresis Methods for the Separation of Intact Heparin and Related Impurities Using Highly Concentrated Buffers with Short Capillaries
Robert Weinberger and T. Wielgos - CE Technologies, Inc., Chappaqua, NY and Baxter Healthcare,

9:40 – 10:00 O#4 Automated Fraction Trapping of Purified Compounds from Preparative Chromatography via a Specialized Polymer Resin Packed within a HPLC Column
Joan Stevens; L. Roenneburg; C. Wingad - Gilson, Inc., Middleton, WI

10:00 – 10:10 Break

10:10 – 10:30 O#5 A Revolutionary Approach to SFC Design
Terry Berger - Aurora SFC Systems, Inc., Sunnyvale, CA

10:30 – 10:50 O#6 At-Column Dilution in Achiral Preparative SFC: Improving Column Performance
Jeff P. Kiplinger and P. M. Lefebvre - Averica Discovery Services, Inc., Worcester, MA

10:50 – 11:10 O#7 HPLC Column ApplRapid Optimization in HPLC Method Development – Exploiting Both Physics and Chemistry
John R. Palmer - Agilent Technologies, Inc., Santa Clara, CA

11:10 – 11:30 O#8 Addressing the Challenges of Melamine Analysis in Infant Formula via GC/MS
Julie Kowalski; M. Misselwitz; J. Cochran - Restek Corporation, 110 Benner Circle, Bellefonte, PA

11:30 – 11:50 O#9 Streamlined Rapid HPLC Method Development for Pharmaceutical Analysis: Plate and Temperature, Strategic and Scientific
Gang Xue; G. Cheng; F. Lestremau; J. Harwood; M. Hana-Brown - Pfizer Global R&D, Groton, CT

11:50 – 12:10 O#10 Strategies for Adjusting to the Worldwide Acetonitrile Shortage
Robert W. Giuffre - Agilent Technologies, Inc., Santa Clara, CA

12:10 – 1:30 Lunch, Poster Session and Vendors' Exhibit

Section 2: New Advances in Chromatographic Separations

Chair: John McCaffrey, Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, CT

1:30 – 2:00 O#11 Plenary Lecture
Reversed Phase Liquid Chromatography: Retention Mechanisms as Studied by Molecular Simulation
Mark R. Schure; J. L. Rafferty; J. I. Siepmann - Rohm and Haas Company, Philadelphia, Pa and Department of Chemistry, University of Minnesota

2:00 – 2:20 O#12 Lipophilicity Determination – Extraction or Liquid Chromatography
Eli Grushka - Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

2:20 – 2:40 O#13 Adsorption of Solutes and Organic Modifiers in Reversed Phase Liquid Chromatography
Attila Felinger - University of Pecs, Pecs, Hungary

2:40 – 3:00 O#14 General Mechanism of Ionic Species Retention in Reversed-Phase HPLC
Yuri Kazakevich and C. Flores - Seton Hall University, South Orange, NJ

3:00 – 3:20 O#15 Peak Tailing of Basic Compounds: The Role of Analyte Interactions at the Surface
Brian A. Bidlingmeyer and R. D. Ricker - Agilent Technologies, Santa Clara, CA

3:20 – 3:30 Coffee Break and Vendors' Exhibit

Section 3: Ultra High Pressure Liquid Chromatography – Memorial Session Dedicated to Marianna Kele

Chair: Uwe Neue, Waters Corporation, Milford, MA

3:30 – 4:00 O#16 Plenary Lecture
UPLC® Principles and Applications
Uwe Neue; B. Bunner; A. Kromidas; P. C. Iraneta; S. Serpa; T. Wheat; B. Gillece-Castro; C. Hudalla - Waters Corporation, Milford, MA

4:00 – 4:20 O#17 High-Speed, High-Resolution UPLC Separation at Zero Degrees Celsius
John R. Engen; T. E. Wales; K. E. Fadgen; G. C. Gerhardt - Northeastern University, Boston, MA

4:20 – 4:40 O#18 Improvements of Peptide and Protein Analysis Utilizing UHPLC Separations
Brenna E. McJury; J. W. Jorgenson – University of North Carolina, Chapel Hill, NC

4:40 – 5:00 O#19 Using Fused-Core Particle Technology to Achieve Near Sub-Two-Micron Performance Using Conventional HPLC Equipment
Thomas J. Waeghe; R. T. Moody; C. L. Zimmerman - MAC-MOD Analytical,

5:00 – 5:20 O#20 Evaluation of sub-2μm Zirconia-PBD Particles for Multi-Modal UHPLC
Dan Nowlan, B. Yan, C. V. McNeff and R. A. Henry - ZirChrom Separations, Anoka, MN

FINAL PROGRAM FOR 9TH CSABA HORVÁTH MEDAL AWARD SYMPOSIUM

Poster Session 1

Tuesday April 28th, 2009

9:00 am to 5:00 pm

P#1
Title: Analysis of Absinthe by Solid Phase Extraction and LC-M/MS: A simple test for thujone concentration
Authors: J. Hackett; M. J. Telepchak; M. J. Coyer
Institution: Northern Tier Research, UCT Inc.,
Contact: jhackett@ntr.bz

P#2
Title: Selective Depletion of Phospholipid Interference Utilizing HybridSPE-PPT Technology
Authors: C. R. Aurand
Institution: Supelco
Contact: bill.panciocco@sial.com

P#3
Title: Evaluation of Operating Conditions in Analytical and Preparative SFC
Authors: L. Miller
Institution: Amgen
Contact: millerl@amgen.com

P#4
Title: Ion Mobility Spectrometry --- Applications in Cleaning Verification and Potential for Pharmaceutical Analysis
Authors: C. Qin, A. Granger, J. McCaffrey, D.L. Norwood
Institution: Boehringer-Ingelheim Pharmaceuticals, Inc.
Contact: cindy.qin@boehringer-ingelheim.com

P#5
Title: Comparison of Chiral Separations Using High Performance Liquid Chromatography and Supercritical Fluid Chromatography on Coated and Immobilized Amylose and Cellulose 3,5-Dimethylphenyl Carbamate Columns
Authors: K. A. Nadeau
Institution: Amgen
Contact: nadeau@amgen.com

P#6
Title: Cationic-Protein Binding to Vesicles: Asymmetric Location of Anionic Phospholipids
Authors: F. Torrens; G. Castellano
Institution: Universitat de València, Spain
Contact: francisco.torrens@uv.es

P#7
Title: Automated Approach to Eliminating the Concentration and Dry Down Bottleneck Associated with Purified Compounds from Preparative Chromatography
Authors: J. Stevens, Ph.D; L. Roenneburg
Institution: Gilson, Inc.
Contact: jstevens@gilson.com

P#8
Title: A Totally Automated System for Purifying Large Quantities of Sample via Shallow Isolation Gradients or Stacked Injections and Fraction Collection
Authors: J. Stevens, Ph.D; L. Roenneburg
Institution: Gilson, Inc.
Contact: jstevens@gilson.com

P#9
Title: Increasing Throughput of Gel Permeation Chromatography (GPC) Clean-up via a Parallel Sample Extraction Process, Collection Window Determination, and Individual Collection per Column
Authors: J. Stevens, Ph.D; M. Halvorson, Ph.D.
Institution: Gilson, Inc.
Contact: jstevens@gilson.com

P#10
Title: Method Development and Validation for the Determination of Gentamicin by HPLC
Authors: J. Cabinian, X. Wang , L. Alquier
Institution: Cordis Corporation
Contact: jcabinia@its.jnj.com

P#11
Title: Newly synthesized stationary phase used on method development for α- and β-emitters measurements in environmental samples
Authors: R. I. Olariu; C. Arsene; D. Humelnicu; M. Duncianu
Institution: "Al.I. Cuza" University of Iasi, Faculty of Chemistry
Contact: oromeo@uaic.ro

P#12
Title: Fast reversed-phase ion-pair liquid chromatography in the simultaneous analysis of nitrite and nitrate in rainwater samples collected in the north-eastern Romania over 2003-2008
Authors: C. Arsene; R. I. Olariu; M. Ponzo; D. Vione
Institution: "Al.I. Cuza" University of Iasi, Faculty of Chemistry
Contact: carsene@uaic.ro

P#13
Title: Investigation of Chiral Separation by GC using Chirasil-Val as Stationary Phase
Authors: Q. Chen; N. Grinberg; S. Ma; J. Lorenz; S. Kapadia; Y. Wang; C. Manolescu; H. Lee; S. Shen; J. McCaffrey; D. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.
Contact: qian.chen@boehringer-ingelheim.com

FINAL PROGRAM FOR 9TH CSABA HORVÁTH MEDAL AWARD SYMPOSIUM

WEDNESDAY APRIL 29TH 2009

7:30 – 8:30 – Registration and Breakfast

Section 4: Chiral Separation

Chair: Eli Grushka - Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

8:30 – 9:00 O#21 Plenary Lecture
Chromatographic and Spectroscopic Studies on the Chiral Recognition of Sulfated β-Cyclodextrin as Chiral Mobile Phase Additive
Nelu Grinberg; S. Ma; S. Shen; N. Haddad; W. Tang; J. Wang; H. Lee; N. Yee; C. Senanayake - Boehringer Ingelheim Pharmaceuticals, Inc.

9:00 – 9:20 O#22 Polysaccharide-based chiral phases provide solutions for chiral HPLC and SFC
Jared W. Benedict; P. K. Dutta – AkzoNobel

9:20 – 9:40 O#23 Development of a 2D-Chiral HPLC/MS/MS Method for PF-03651639, its Enantiomer and a Metabolite
Yizhong Zhang - Pfizer Inc.

9:40 – 10:00 O#24 A Screening Approach for Chiral Method Development Using Reversed Phase Gradient Elution and Immobilized Polysaccharide Stationary Phases
Stanislaw Babiak; H. Wang; R. Thompson; E. Loeser; P. Drumm - Chemical and Analytical Development (CHAD), Novartis Pharmaceuticals Corporation, East Hanover, NJ

10:00 – 10:20 O#25 Enantiomeric Separation Using High Temperature Liquid Chromatography and Chiral Mobile Phase Additives
Shengli Ma; S. Shen; H. Lee; N. Yee; C. Senanayake; N. Grinberg - Boehringer Ingelheim Pharmaceuticals, Inc.

10:20 – 10:40 O#26 Enantiomer Recognition of Amino Acids with (+)18C_6H_4 using Reversed-Phase Liquid Chromatography and Nuclear Magnetic Resonance Spectroscopy
Zhaoxia Liu; R. Thompson; M. Lin; S. Shah; D. Drinkwater; P. Drumm - Novartis Pharmaceuticals Corporation, East Hanover, NJ

10:40 – 10:50 Break

Section 5: LC-MS and Detection

Chair: Heewon Lee - Boehringer Ingelheim Pharmaceuticals, Inc.

10:50 – 11:20 O#27 Plenary Lecture
A Major New Application for Chromatography, Electrophoresis and Mass Spectrometry: Biosimilars
Barry L. Karger - Barnett Institute, Northeastern University, Boston, MA

11:20 – 11:40 O#28 Proteomics as a tool for optimization of human plasma protein separation and characterization of preparations of plasma-derived therapeutic proteins
Djuro Josic - Brown University and Rhode Island Hospital

11:40 – 12:00 O#29 Mass defect: the power of separating peptide fragment ions in a mass spectrum
Yu Shi; X. Yao - Department of Chemistry, University of Connecticut

12:00 – 12:20 O#30 Electrochemical Detection In Bioanalysis: Carbohydrates and Beyond
William R. LaCourse - University of Maryland, Baltimore County

12:20 – 1:30 Lunch, Poster Session and Vendors' Exhibit

1:30 – 2:00 Award Presentation to Professor Georges Guiochon – University of Tennessee, Knoxville, TN

2:00 – 2:40 O#31 Csaba Horváth Lecture

Performance of HPLC columns packed with Very Fine Particles. Consequences of the heat effect
Georges Guiochon; F. Gritti; K. Kaczmarski - Department of Chemistry, University of Tennessee, Knoxville, TN

2:40 – 3:00 Coffee Break and Vendors' Exhibit

Section 5: LC-MS continues

Chair: Nelu Grinberg - Boehringer Ingelheim Pharmaceuticals, Inc.

3:00 – 3:20 O#32 Development and Validation of HPLC Stability Indicating Methods Using Charged Aerosol Detection Technology
Michael Swartz and M. Emanuele - Synomics Pharmaceutical Services, LLC, Wareham, MA

3:20 – 3:40 O#33 Genotoxic Impurity Control in Drug Substance Development
Heewon Lee; S. Shen; S. Ma; N. Grinberg - Boehringer Ingelheim Pharmaceuticals, Inc.

3:40 – 4:00 O#34 Development of an HPLC-MS Method for Tracking Specific LBPA Variants in Biological Matrices in Response to Drug-Induced Phospholipidosis
Vladimir V. Papov Jr; K. N. Locke; A. M. Mineo; B. Johnson; Z. Huang; J. H. Stoltz; S. Jayadev; J. A. Phillips - Boehringer Ingelheim Pharmaceuticals

Concluding Remarks – Nelu Grinberg

Poster Session 2

Wednesday April 29th, 2009

9:00 am to 5:00 pm

P#14
Title: Use of 2-mm I.D. C18 Monolithic Chromatography in a Discovery Setting: A Means for the 3X-Reduction of Cycle Times
Authors: J. M. Hollembaek, B. J. Rago, A.King-Ahmad, K. F. Wilkinson, C.L. Holliman
Institution: Pfizer Global R & D
Contact: john.hollembaek@pfizer.com

P#15
Title: Effect of Ionic Liquid Anions on the Retention of Naphthalene Derivatives in SFC
Authors: Y. Wang; N. Grinberg; Q. Chen; C. Manolescu; J. McCaffrey; D. L. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals
Contact: yuwen.wang@boehringer-ingelheim.com

P#16
Title: Research and Testing of Biodiesel, a State of Connecticut Alternate Energy Initiative
Authors: J. D. Stuart; R. S. Parnas; C. R. Perkins
Institution: University of Connecticut
Contact: james.stuart@uconn.edu

P#17
Title: UPLC and RRLC with the Corona ultraTM Charged Aerosol Detector
Authors: M. Plante; C. Crafts, B. Bailey; I. Acworth
Institution: ESA Biosciences
Contact: kcourtemanche@esainc.com

P#18
Title: Electrochemistry On-line with LC-MS to Produce and Characterize Drug Metabolites and Degradants
Authors: I. Acworth
Institution: ESA Biosciences
Contact: kcourtemanche@esainc.com

P#19
Title: AFFF/MALS and Ion Mobility for Analysis of Antibodies and Non-Covalent Complexes
Authors: B. A. Andrien, Jr.
Institution: Alexion Pharmaceuticals
Contact: andrienb@alxn.com

P#20
20. Title: HPLC Enantiomeric Separation of an Aromatic Amine using Crown Ether Tetracarboxylic
Authors: S. Shen; S. Ma; H. Lee; N. Yee; C. Senanayake; N. Grinberg
Institution: Boehringer-Ingelheim Pharmaceuticals, Inc.
Contact: sherry.shen@boehringer-ingelheim.com

P#21
Title: Practical Application of Fused-Core® Columns with Standard HPLC Instrumentation: Understanding the Importance of Extracolumn Volume
Authors: T. J. Waeghe; R. T. Moody; C. L. Zimmerman
Institution: MAC-MOD Analytical
Contact: twaeghe@mac-mod.com

P#22
Title: An HPLC Method Using Charged Aerosol Detection for the Quantitative Determination of Methylsulfonyl Piperidine Analyte Lacking a UV Chromophore
Authors: M. Jerfy; A. Soman
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.
Contact: madhuri.jerfy@boehringer-ingelheim.com

P#23
Title: STRUCTURE ELUCIDATION OF AN PROCESS IMPURITY IN A P38 MAP KINASE INHIBITOR DRUG SUBSTANCE USING LC/MS AND NMR
Authors: F. Qiu; S. Campbell; A. Granger; Z. Tan; J. Song; D. L. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.
Contact: fenghe.qiu@boehringer-ingelheim.com

P#24
Title: Retained Diluent Chromatography: A Revolutionary New Variation of HPLC
Authors: E. Loeser; S. Babiak
Institution: Novartis Pharmaceuticals Corporation
Contact: stanislaw.babiak@novartis.com

P#25
25. Title: Gravimetric Measurement of Excess Adsorption in a Binary Solvent System
Authors: E. Loeser; S. Babiak; Z. Liu
Institution: Novartis Pharmaceuticals Corporation
Contact: stanislaw.babiak@novartis.com

P#26
Title: Utilization of UHPLC in Pfizer Groton
Authors: C. G. Cheng
Institution: Pfizer Inc
Contact: guilong.cheng@pfizer.com

P#27
Title: Utilizing Fused-Core Technology for LC-MS Applications
Authors: Hillel K. Brandes, Craig R. Aurand, David S. Bell, Richard A. Henry, Wayne Way, Russel Gant, and Paul Ross
Institution: Supelco, Div. of Sigma-Aldrich, Bellefonte, PA 1682301
Contact: bill.panciocco@sial.com

ORAL PRESENTATION ABSTRACTS

Session 1: Separation Techniques

O#1

Title: Some Insights on Retention and Selectivity for Hydrophilic Interaction Chromatography

Authors: R. Thompson & M. Yang
Institution: Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080 USA
Contact: richard.thompson@novartis.com

Using a pure silica stationary phase along with a simple acetonitrile – phosphoric acid aqueous mobile phase provide a powerful and versatile tool for the analysis of a broad range of polar compounds such as amino acids and pharmaceutical compounds. The retention of solutes is influenced by the nature of the stationary phase, the organic solvent and the aqueous mobile phase components. The retention mechanism is predominantly electrostatic interactions occurring between the solute and stationary phase. Selectivity for cationic or zwitterionic solutes is largely due to differences in their ion exchange capabilities, and consequently influenced by the pKa and lipophilicity of the solutes.

O#2

Title: Pushing the envelope on separation speed: "knowing when to push it and when to back off"

Author: Mark J. Hayward
Institution: Lundbeck Research USA - 215 College Rd. - Paramus, NJ 07652
Contact: MHAY@Lundbeck.com

In Knox and Saleem's 1969 discussion on the potential of fast LC, they predicted the use of columns less than 1 cm long packed with 1 micron diameter particles producing 5000 plate separations in 20 seconds (1). This made perfect sense four decades ago when the separation performance was primarily mass transfer limited because particle diameters were greater than 50 microns. Since the mass transfer and hence separation efficiency would be expected to be inversely proportional to the square of particle diameter, it was naturally assumed that as particle diameter was decreased, column length would also would reduced such that the flows and pressures would be fairly similar to those already demonstrated or in use. The result of this pursuit would be essentially the same separations as before, but much faster due to the short column, small particle combination. However, despite four decades of significant effort, Knox and Saleem's prediction has not been achieved because we have not been able to shorten the column to 1 cm without paying a severe price in the separation.

Now, 40 years later, our view is different. We have an abundance of small particle columns (less than 10 micron) and we find that reverse phase separations with good columns are not particularly mass transfer limited with 5 micron particles (2, 3). Now, optimized LCs are often operated where the column performance readily exceeds that of the instrument and most (80%) of the observed dispersion occurs outside the column (3). The effect is that reducing particle diameter in order to improve mass transfer eventually doesn't have the predicted effect (4) and instead has very limited benefit. Ultimately, at the extremes, smaller particles actually slow the separation (5), giving less resolution per unit time, because high pressures force the use of lower eluent velocity.

Clearly, there are trade-offs that must be made when pushing the reverse phase LC speed envelope since any of the extremes generally force sacrifices in either speed, separation efficiency, or both. This presentation describes a systematic empirical approach where a set of column dimensions (column ID as well as particle diameter varied) are evaluated in the context of the pressure and flow limitations for a variety of instruments. The goal of the evaluation is to maximize velocity without sacrificing resolution. Furthermore, the interplay of the use of temperature control is explored to see what boundaries (if any) are further imposed with this important parameter. The practical results show that once the injection process (autosampler) is properly matched with column ID, the higher separation speeds are generally achieved at moderate values in other dimensions. Solvent choices as well as mode of operation (gradient vs. isocratic) play prominent roles in achieving the highest speeds. Finding fast conditions is most readily facilitated by maximizing velocity while using temperature control as the primary optimization tool.

1. J.H. Knox, M. Saleem, J. Chromatogr. Sci. 1969, 7, 614.
2. R.P.W. Scott, J. Liq Chromatogr Related Tech. 2002, 25(17), 2567.
3. F. Gritti, A. Felinger, G. Guiochon, J. Chromatogr. A 2006, 1136, 57.
4. M.J. Wirth, J. Chromatogr. A 2007, 1148, 128.
5. T.L. Chester, S.O. Terami, J. Chromatogr. A 2005, 1096, 16.

O#3

Title: Evolution of Capillary Electrophoresis Methods for the Separation of Intact Heparin and Related Impurities Using Highly Concentrated Buffers with Short Capillaries

Authors: R. Weinberger and T. Wielgos
Institution: CE Technologies, Inc. and Baxter Healthcare
Conract: robertweinberger@aol.com

Contamination of heparin with oversulfated chondroitin sulfate (OSCS) drove the development of capillary electrophoretic methods to ensure the integrity of the world's supply. The official method used 36 mM sodium phosphate, pH 3.5 in a 50 um capillary. The first round of method improvements used 600 mM sodium phosphate pH 3.5 as the background electrolyte in a 25 um capillary. Lowering the pH to 2.5 improved the resolution permitting the use of a shortened capillary. The role of the buffer counterion was investigated as well. Switching to lithium allowed a 40% increase in voltage further speeding the separation. This lecture will review the roots of the heparin crisis and describe the evolution of methodology for the separation of intact glycosoaminoglycosides. Using the short-end of the capillary, a 4 minute high resolution separation was possible with limits of detection far below 0.1% for OSCS.

O#4

Title: Automated Fraction Trapping of Purified Compounds from Preparative Chromatography via a Specialized Polymer Resin Packed within a HPLC Column

Authors: J. Stevens, Ph.D; L. Roenneburg; C. Wingad
Institution: Gilson, Inc.
Contact: jstevens@gilson.com

Preparative chromatography is the designated purification technique employed for pharmaceutical, nutraceuticals and biologically compounds, showing possible activity. It is imperative that a quantity of purified compound be available for activity assays, additional synthesis and storage. Researchers work extremely hard to create an active compound; however there is always an impurity or non-reactive component that the FDA requires to be isolated, removed or defined. Studies have shown that synthetic scale up can create impurities or additional bi-products that were not observed at an analytical scale compromising the purity of the active compound. Therefore preparative chromatography is employed to separate and isolate the active compound. On average when semi-preparative chromatography is employed (e.g. 21.1 x 50 mm, column geometry, flow rate 15-50 mL/min, compound mass 5-300 mg) the average volume associated with the purified fraction is approximately 5-10 mL. Scaling the purification to a preparative level (e.g. > 50 x 150 mm, column geometry, flow rate > 75 mL/min, compound mass 0.3 – 15 gm) creates a fraction volume 2 to 10 times the fraction volume stated above (e.g. 20-120 mL). The purified fraction collected requires obviously dry down to remove the mobile phase. Since the common preparative technique employed for purification is RP HPLC the purified fraction will contain an aqueous and organic component including a modifier (e.g. TFA, TEA, formic acid). Elimination of the solvent associated with the purified fraction requires a vacuum and a temperature increase. Concentration of the mobile phase modifier has been shown to cause hydrolysis of functional groups, possibly destroying the activity of the purified compound. In addition evaporation or dry-down is time consuming (e.g. 8-24 hrs) and requires manual intervention. Employing an activated media packed in a SPE cartridge the automated system can intelligently identify the purified fractions, and concentrate the purified compound onto the SPE cartridge, eliminating all the possible caveats and limitations regarding preparative purification. This approach allows over 30 purified fractions/compounds to be processed in less than 2 hours without manual intervention. General cost savings is approximately $5,000/week based on research/instrumentation reduction.

O#5

Title: A Revolutionary Approach to SFC Design

Authors: T. A. Berger
Institution: Aurora SFC Systems, Inc
Contact: tberger@aurorasfc.com

A revolutionary approach to SFC instrumentation preconditions the fluid before it is metered by a conventional HPLC pump. The HPLC hardware and software are virtually unchanged. Conversion between HPLC and SFC is simple and fast. Since the SFC module pre-compresses the fluid the HPLC pump compressibility can be set to zero. Since the pump performs no compression, there is no heat of compression, and almost no flow noise. These characteristics dramatically decrease UV detector noise and improved dynamic range. SFC detection limits similar to HPLC are now achievable. This 20 x improvement has broad implications for routine analysis, EE determinations, chiral metabolites, etc.
This instrumental approach allows one to convert older HPLC to higher performing SFC's. The 3 to 5 times higher diffusion coefficients, and 3 to 5 x lower viscosity allows these older conventional HPLC's and standard silica based columns to achieve similar speed to UHPLC but at significantly lower pressures.

O#6

Title: At-Column Dilution in Achiral Preparative SFC: Improving Column Performance

Authors: J. P. Kiplinger; P. M. Lefebvre
Institution: Averica Discovery Services Inc, Worcester, MA
Contact: jeff.kiplinger@avericadiscovery.com

In the 1990s, the use of reversed phase gradients on C_{18} media in short column formats as 'generic separation methods' developed in critical applications such as analysis of crude synthetic isolates, in-vitro drug metabolism assays, and purification of drug discovery leads. Reversed-phase gradient HPLC is now frequently used for purification. A common approach is to inject up to 100 mg of crude synthesis product on a 20 x 50 mm 5µ C_{18} column and elute with a fast steep gradient of water and acetonitrile, usually with an organic acid or buffer modifier. MS detection (or UV detection after LCMS analysis) ensures that the desired peak is collected.

Because in a generic approach compounds must be easily loaded on column no matter what their solubility, many analysts use a procedure generally known as "At-Column Dilution" in which the sample in a strong diluent (e.g. DMSO/methanol) is added slowly to mobile phase to simulate the initial (high aqueous) condition.

We are exploring the adaptability of normal phase chromatography such as SFC to the purification of achiral library synthesis products by 'generic' separation methods. As part of this work we are interested in methods that improve column loadability and performance. In this work, at-column dilution is investigated in a gradient SFC purification application.

O#7

Title: HPLC Column Appl Rapid Optimization in HPLC Method Development – Exploiting Both Physics and Chemistry

Authors: J. R. Palmer
Institution: Agilent Technologies, Inc.
Contact: john_palmer3@agilent.com

The most important aspect of any HPLC method development scheme is to achieve adequate resolution among all analytes. Following that, optimization for speedy assays to better manage lab resources becomes the important goal. While many chromatographers approach these tasks separately, it is much more efficient to pursue both goals simultaneously. The new generation of smaller dimension columns that maintain resolving power by utilizing smaller diameter particles make physical speed choices easier than ever. This seminar will present a simple to follow pathway to quicker method development. We will demonstrate how to quickly evaluate the effects of the separation parameters pH, isocratic organic composition, gradient optimization and temperature to provide the best overall method. Attendees will leave this meeting with a systematic approach to method development that should reduce the time needed to produce rugged, robust HPLC methods. The goal is a better method faster – not just a faster method!

O#8

Title: Addressing the Challenges of Melamine Analysis in Infant Formula via GC/MS

Authors: J. Kowalski; M. Misselwitz; J. Cochran
Institution: Restek Corporation
Contact: julie.kowalski@restek.com

Recent reports linking the presence of melamine in pet food and infant formula to illnesses and deaths have led to the recall of a wide variety of tainted food products and calls for stricter product testing. Melamine is not considered toxic alone at low doses; however, the observed toxicity was attributed to melamine exposure in the presence of cyanuric acid. In combination, these compounds form insoluble crystals in the kidneys, causing illness and eventual renal failure. Melamine is not a legal food additive; it is a nitrogen-rich industrial compound used for plastics, flame-resistant products, and some cleaning agents. However, melamine and related byproducts are sometimes added illegally to food products in order to falsely represent the amount of protein present, since protein level in many products is determined using nonspecific assays for nitrogen content.

Melamine analysis serves as an excellent example to discuss the challenges associated with trace analysis in difficult matrices. Several analytical challenges exist including the need to analyze different matrices, low levels of detection and the chemical similarity to common matrix components. Several methods based on GC/MS and LC/MS/MS have been published, however, challenges remain for the analyst. GC/MS methodology will be the main focus because instrumentation is more prevalent. The following work was performed to establish conditions for melamine analysis down to 1µg/g in infant formula using GC/MS. The use of solvent standards and matrix matched standards will be discussed in reference to the matrix effect that can alter the intensity of a given analyte, yielding false positives and sometimes false negatives. In addition, the matrix protection effect will be demonstrated. Matrix protection effects result when the matrix enhances the signal of an analyte.

O#9

Title: Streamlined Rapid HPLC Method Development for Pharmaceutical Analysis: Plate and Temperature, Strategic and Scientific

Authors: G. Xue; G. Cheng; F. Lestremau; J. Harwood; M. Hana-Brown
Institution: Pfizer Global R&D
Contact: gang.xue@pfizer.com

As main workhorse for pharmaceutical analysis, high performance liquid chromatography (HPLC) consumes considerable amount of resources in R&D analytical lab on its method development. The latest developments in high efficiency instrument, column chemistry and prediction software made it possible to significantly expedite the process strategically and scientifically.

Instead of ransacking the variety of column chemistry for optimal selectivity, for most achiral separations, we turn to the maximized plate number to brutal-forcedly achieve the required resolution. In addition, temperature is utilized in the process to tweak the selectivity and run time. With this approach, we are able to adopt a strategy of three orthogonal generic UHPLC methods followed by six injections of temperate and gradient screening to achieve robust purity methods. A suite of ACD Labs software are successfully applied to multiple stages to simulate and optimize separation with the acquired empirical data, which further reduces the amount of lab work and speed up development cycle. With this strategy, most drug development projects require less than one day turn-around time.

O#10

Title: Strategies for Adjusting to the Worldwide Acetonitrile Shortage

Authors: R. W. Giuffre
Institution: Agilent Technologies
Contact: bob_giuffre@agilent.com

Chromatographers around the world are trying to adjust to the worldwide shortage of one of the most used solvents in high pressure liquid chromatography: acetonitrile. This solvent with its excellent properties for this technique has been THE organic solvent of choice when developing methods for HPLC.
The shortage of acetonitrile has forced chromatographers to reevaluate both existing methods and methods that are currently under development. Even more worrisome is that methods used throughout the pharmaceutical industry are validated and cannot be changed without extensive regulatory hurdles being overcome.
This paper will examine approaches that may be considered including:
- Changes to the column dimensions and particle size
- Permitted changes to validated methods as discussed by the USP and FDA
- Alternate solvents that may be considered with a discussion of isoelutropic strengths and viscosities of those solvents.

Session 2: New Advances in Chromatographic Separation

O#11

1. Title: Reversed phase liquid chromatography: Retention mechanisms as studied by molecular simulation

Authors: Mark R. Schure[1], Jake L. Rafferty[2], and J. Ilja Siepmann[2,3]
Institution: [1]Theoretical Separation Science Laboratory, Rohm and Haas Company, Springhouse, PA [2]Department of Chemistry, University of Minnesota, Minneapolis, MN
[3]Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN
Contact: mark.schure@gmail.com

One of the unresolved mysteries in separation science is the retention mechanism(s) of reversed phase liquid chromatography (RPLC). Given the complexity of RPLC systems, it appears that a true molecular theory or complete thermodynamic treatment of chromatography is an unreachable goal and, maybe, also a fruitless exercise. Starting with pioneering efforts in the 1970s, molecular simulations have become the statistical mechanical method of choice for the description of liquid systems where chemical specificity is important. Since liquid chromatography is just a special case of a multicomponent liquid system at a complex interface, it is no surprise that molecular simulation should bring a high level of understanding and quantitative prediction to the RPLC problem.

Recent work using advanced molecular simulation techniques (configurational-bias Monte Carlo simulations in the Gibbs ensemble using accurate and transferable force fields) has provided a comprehensive picture of the retention mechanisms for small polar and non-polar molecules and polycyclic aromatic hydrocarbons in RPLC. These studies, which show excellent agreement with experimental retention data, yield precise information on the solute distribution and the conformation of the bonded-phase chains on the surface of the chromatographic support.

A complex distribution is found for the solvent components of water: methanol and water: acetonitrile mixtures near the substrate, in the interior region of the bonded-phase chains, and at interface of retentive and mobile phases. Comparison

to bulk *n*-hexadecane phases demonstrates that the complex geometry of the retentive phase prevents the use of a solubility theory to determine the retention of solute molecules. Using an ideal gas phase reference state, the simulations allow for a clear assessment of the driving forces for retention in RPLC. Molecular simulations allow for an analysis of the system through snapshots, spatially resolved distribution profiles, and various parameters of chain conformation, without requiring the use of many equations in this talk.

O#12

Title: Lipophilicity Determination – Extraction or Liquid Chromatography
Authors: E. Grushka
Institution: Institute of Chemistry, The Hebrew University of Jerusalem
Contact: eliga@chem.ch.huji.ac.il

The bioactivity of a new drug candidate can be estimated from its lipophilic nature. Therefore, lipophilicity measurements are important in drug discovery procedures. Traditionally, lipophilicity is determined by measuring the partition of a drug candidate in n-octanol-water extraction system. The n-octanol-water partitioning system is essentially a two-phase shake-flask extraction system. While the shake-flask is experimentally very simple, there are several inherent difficulties in this technique. Among these difficulties are (1) the need for a relatively large amount of the compound to be measured, (2) the need for high purity of that compound, (3) difficulties in phase separation after the extraction process and (4) the inability to measure accurately log P for highly hydrophobic or hydrophilic substances. Liquid chromatography does not suffer from the above limitation and since both techniques are based on thermodynamically-driven partitioning of solutes between two phases, liquid chromatography can be used instead of the n-octanol-water system to determine lipophlicities. A large number of papers are devoted the correlation between the chromatographic data, chiefly trying to relate the retention parameter k' to log P. However, in chromatography the two phases (mobile and stationary) are not as well defined as in extraction. For example, in a reversed-phase column the stationary phase can include at the same time alkane chains, silanol groups and extracted components of the mobile phase. As a result, the agreement between the chromatographic log k' data and the shake-flask log P values is often not as good as expected. In this presentation we will compare several types of stationary phases in order to choose a chromatographic system that best emulates the n-octanol-water system. We examine the contribution of the various stationary phases to the retention process and, therefore to log k'. Using the Abraham linear salvation energy relationships (LSER) we compare the chromatographic log k' data, obtained using several column modifications, with the log P values. Through LSER, we can investigate the effect of changing operating conditions on the various interactions between the solutes and both the mobile and stationary phases. The comparison of the LSER system constants for the two techniques points out to the column configuration that best mimics the results obtained with the shake-flask method.

O#13

Title: Adsorption of solutes and organic modifiers in reversed phase liquid chromatography
Authors: A. Felinger
Institution: University of Pecs, Hugary
Contact: felinger@ttk.pte.hu

Retention in reversed phase liquid chromatography is strongly influenced by the adsorption of the organic modifier on the stationary phase. The structure of the hydrophobic ligands, embedded polar groups, etc. has an important effect on the retention of solute molecules. The adsorption isotherms of common organic modifiers were measured using the inverse method on a number of silica based C18 packed columns. The effects of bonding density and endcapping on the adsorption properties were determined. Investigations were done by analyzing the shapes of nonlinear perturbations of solvents, measured with a refractive index detector from a column in equilibrium with pure water. The solvent adsorption has been modeled by single Langmuir isotherm and by excess isotherms. The results are in good agreement with available data on solvent adsorption obtained with the minor disturbance method.

The adsorption isotherms of a number of neutral and basic analytes have been determined on a series of endcapped and non-endcapped columns with frontal analysis and the variation of the isotherm parameters against the surface coverage of the organic ligands are used as a means to characterize the surface of the hydrophobic stationary phases.

O#14

Title: General Mechanism of Ionic Species Retention in Reversed-phase HPLC
Authors: Y. Kazakevich; C. Flores
Institution: Seton Hall University, South Orange, NJ
Contact: kazakeyu@shu.edu

HPLC retention of ionic and ionizable analytes is known to be dependent on the mobile phase pH, type of buffer and salt concentration. The influence of the salt type and concentration has been attributed to so-called “chaotropic” mechanism and associated to “chaotropic” and “cosmotropic” properties of inorganic ions known as Hoffmeister series [1]. This mechanism was originally exclusively related to the ability of these ions to disrupt the solvation sell of ionic analyte, thus expose analyte’s inherent hydrophobicity for interaction with the stationary phase.

Further studies of the influence of organic modifier on the retention of ionic species demonstrated principal difference in salt effect on ionic analyte retention between methanol and acetonitrile. The ability of acetonitrile to form thick adsorbed layer on the adsorbent surface and it's high pi-electron density create a favorable environment for the retention of inorganic ions with significant charge delocalization, which result in significant retention increase of analyte possessing opposite charge. While similar effect completely eliminated when acetonitrile is substituted with methanol.

Our recent studies have demonstrated that the influence of different inorganic ions does not influence charged analyte retention in the similar sequence as the position of these ions in Hoffmeister series. In acetonitrile/water mobile phases ALL ions exhibit "chaotropic" effect, even the ions known to have "cosmotropic" properties (favorable for the formation of solvation shell) show the increase of the analyte retention with the increase of their concentration.

Retention mechanism based on the pi-pi-specific interactions in the presence of acetonitrile in the mobile phase will be discussed.

O#15

Title: Peak Tailing of Basic Compounds: The Role of Analyte Interactions at the Surface

Authors: B. A. Bidlingmeyer; R. D. Ricker
Institution: Agilent Technologies
Contact: brian_bidlingmeyer@agilent.com

Retention of basic compounds (+ charge) are retained longer on bonded phases at pH 7 due to the electrostatic interactions with the unreacted, ionized silanols (- charge). It is generally believed that this electrostatic interaction is also responsible for tailing. Further, it is generally believed that at low pH peaks tail less than at pH 7 due to ion suppression of silanols. However, we have observed that this is not always the case. Peaks do have less retention and appear sharper; but, in actuality, tailing can exist at pH 2 and tailing can be lower at pH 7. Why is this? This work investigates the observations made at pH 2 & 7 and suggests a possible explanation as to why the peak shapes are more tailed at pH 2.

Additionally, observations of compound-specific tailing at pH 7 suggests that attempting to use a single probe to predict tailing behavior of all basic compounds may be a futile effort. A discussion of the role of the analyte in contributing to tailing will be presented.

Session 3: Ultra High Pressure Liquid Chromatography – Memorial Session dedicated to Marianna Kele

O#16

Title: UPLC® Principles and Applications

Authors: U. D. Neue; B. Bunner; A. Kromidas; P. C. Iraneta; S. Serpa; T. Wheat; B. Gillece-Castro; C. Hudalla
Institution: Waters Corporation, 34 Maple St., Milford, MA 01757, USA
Contact: Uwe_Neue@waters.com

Both our understanding of the UPLC® technology and the application areas covered continue to increase. From a fundamental standpoint, the knowledge of the influence of pressure on retention continues to increase. The origins and magnitudes of thermal effects are also understood better with improved models. We will show both effects from the standpoint of theory and practice. In addition, we will demonstrate practical improvements in column performance and permeability. Furthermore, new application areas are being explored, from protein and peptide separations to HILIC applications from food sugars to glycans.

O#17

Title: High-Speed, High-Resolution UPLC Separation at Zero Degrees Celsius

Authors: J. R. Engen; T. E. Wales; K. E. Fadgen; G. C. Gerhardt
Institution: Northeastern University
Contact: j.engen@neu.edu

Although elevated temperatures are known to improve chromatographic efficiency, some chromatographic applications require low temperatures where chromatographic efficiency is generally poor. One method that requires low-temperature separations is hydrogen/deuterium exchange mass spectrometry (HXMS). In order to maintain the deuterium label on proteins and peptides, chromatographic separation in HXMS must be done rapidly (usually in under 8-10 minutes) and at zero degrees Celsius. Traditional RP-HPLC with ~3 micron particles has shown generally poor chromatographic performance under these conditions. Ultra performance liquid chromatography (UPLC) employs particles smaller than 2 microns in diameter to achieve superior resolution, speed, and sensitivity as compared to HPLC, even at low temperature. Here we present construction and validation of a custom UPLC system for HXMS. The system is based on the Waters nano ACQUITY platform and contains a Peltier-cooled module that houses the injection and switching valves, online pepsin digestion column, and C-18 analytical separation column. Single proteins in excess of 95 kDa and a four-protein mixture in excess of 250 kDa have been used to validate the performance of this new system. Near baseline resolution was achieved in 3-6 minute separations of peptides at 0 °C. The median chromatographic peak width in such separations

was ~2.7 sec at half height. Deuterium recovery was similar to that obtained using a conventional HPLC and ice bath. This new UPLC system represents a significant advancement in HXMS technology that is expected to make the technique more accessible and mainstream in the near future.

O#18

Title: Improvement of peptide and Protein Analysis utilizing UHPLC separations.
Authors: B. E. McJury: J. W. Jorgenson
Insitution: UNC-Chapel Hill, North Carolina
Contact: mcjury@email.unc.edu

Ultra High Pressure Liquid Chromatography began over a decade ago as the desire to pack columns with particles of decreasing size while maintaining column length. Initially, work was done using 1.0 and 1.5 *μ*m non-porous packing material. This packing material was advantageous due to relative ease with which particles with narrow size distributions could be prepared. Although columns packed with small non-porous particles performed well for the analysis of small molecules, they suffered from limited sample loading capacity which prevented the optimal coupling to mass spectrometry with good signal to noise for peptide and protein analysis. Porous particles were then examined due to their 40-fold increase in sample loadability when compared to non-porous particles, which greatly improved the mass spectrometric detection of these compounds. Protein analysis under HPLC conditions has suffered from protein ghosting effects, as well as decreased recovery. Previous work in our lab has shown that analyzing proteins at up to 3,000 bar, the pressure necessary to run long capillary columns packed with sub-2.0 *μ*m particles, has improved both of these issues. The improvements in both peptide and protein analysis as a result of UHPLC were applied to the multidimensional analysis of cell lysates. Both UHPLC and HPLC analyses were performed. While the UHPLC analysis provided superior separations, the off-line analysis proved to be time-consuming and cumbersome when compared to the on-line analysis feasible with standard HPLC systems. Possibilities for combing the resolving power of UHPLC separations with the on-line capabilities of the lower pressure HPLC will be discussed as well.

O#19

Title: Using Fused-Core Particle Technology to Achieve Near Sub-Two-Micron Performance Using Conventional HPLC Equipment
Authors: T. J. Waeghe; R. T. Moody; C. L. Zimmerman
Institution: MAC-MOD Analytical
Contact: twaeghe@mac-mod.com

With the recent downward trends in financial markets and tightening capital budgets, laboratory managers and researchers are seeking other ways of improving instrument and personnel productivity by achieving fast and high-resolution separations that deliver high quality results with improved instrument uptime. As a result, major equipment purchases have been delayed or deferred indefinitely. Fortunately, the development and commercialization of columns packed with superficially porous, 2.7-micron Fused-Core particles have now made it possible to meet the needs for improved throughput and resolution at the more modest pressures (40-50% of sub-two-micron columns) of 400- and 600-bar HPLC systems.

In this presentation we will briefly review the history of superficially porous particles, and some of the published findings on the performance of Fused-Core particles and HALO® columns. We'll examine extracolumn band broadening and dispersion, and its impact on the performance of high efficiency, low volume columns. The various instrument and method parameters that contribute to extracolumn band dispersion will be highlighted, and results of experiments carried out to assess the importance of those parameters will be presented. Some examples of Fused-Core column performance that can be achieved with minor instrument and method modifications will be shown for conventional equipment such as Agilent 1100 and Waters Alliance systems.

O#20

Title: Evaluation of sub-2µm Zirconia-PBD Particles for Multi-Modal UHPLC
Authors: D. Nowlan, B. Yan, C. V. McNeff and R. A. Henry.
Institution: ZirChrom Separations, Inc. 617 Pierce St., Anoka, MN 55303
Contact: danielnowlan@zirchrom.com

Recent advances in HPLC particles and instrument design have created a very strong trend toward the use of columns containing particles smaller than 3µm. HPLC with very small particles has been given the name ultra high performance liquid chromatography, abbreviated as UHPLC. While the door has been opened to higher efficiency, resolution, peak capacity and separation speed, the debate is already beginning to shift back to the familiar topic of column selectivity and the need for new phases on these smaller HPLC particles.

This talk will present data with sub-2µm zirconia coated with polybutadiene (Zr-PBD). The performance of short columns prepared with these new particles will be shown for standards and several other compound classes. The origin of unique

multi-mode selectivity for Zr-PBD columns will be reviewed. While the majority of UHPLC data has been shown for silica-based particles and phases, there can be significant advantages to using substrates that are highly stable toward extremes of pH, temperature and pressure. The pros and cons of using coated zirconia particles will be discussed.

Section 4: Chiral Separation

O#21

Title: Chromatographic and Spectroscopic Studies on the Chiral Recognition of Sulfated β-Cyclodextrin as Chiral Mobile Phase Additive

Authors: N. Grinberg; S. Ma; S. Shen; N. Haddad; W. Tang; J. Wang; H. Lee; N. Yee; C. Senanayake
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.
Contact: nelu.grinberg@boehringer-ingelheim.com

A fast enantiomeric separation of a chiral aromatic amine was achieved, using ultra high pressure liquid chromatography and highly sulfated beta-cyclodextrin (S-β-CD) as a chiral additive in the mobile phase. The stationary phase consisted of a core shell support with a particle size of 2.7 μm. Under these conditions the base-line separation was obtained within 2.5 minutes. The influence of the concentration of the additive, along with the thermodynamics of the separation were studied. Vibrational circular dichroism spectroscopy (VCD) was applied to assess the absolute configuration of the two enantiomeric analytes, as well as the interaction of these enantiomers with the S-β-CD. The VCD results revealed that S-β-CD undergoes a temperature induced conformational change. Further, VCD experiments indicate that the interactions of the two enantiomers with the S-β-CD occur through an inclusion of the aromatic part of the analyte, as well as through electrostatic interaction between the protonated amine and the sulfate groups located at the narrow part of the S-β-CD. Molecular mechanics calculations performed according to the VCD results are consistent with experimental data, providing further evidence of these interactions.

O#22

Title: Polysaccharide-based chiral phases provide solutions for chiral HPLC and SFC.

Authors: J. W. Benedict; P. K. Dutta
Institution: AkzoNobel
Contact: jared.benedict@akzonobel.com

The area of chiral chromatography is a fast growing segment within the HPLC and SFC area. There are still large potential for improvements by developing new chiral stationary phases enabling enhanced resolution and shorter analysis time. In the analytical area, chiral phases with improved efficiency and selectivity giving better resolution and shorter analysis time are desirable. Whereas for preparative purifications, loadability and selectivity are the most important parameters, giving higher productivity and lower purification cost.
This presentation shows how a new generation polysaccharide-based chiral stationary phase expands the possibilities for analytical and preparative chiral chromatography. The silica is based on an in house developed wide pore matrix and coated with a functionalized natural polymer selector. Some inherent and unique qualities of the new media include high resolution, excellent selectivity, no pressure limits and stable performance when switching between compatible mobile phases. Examples of HPLC and SFC applications will be shown along with ways to improve the efficiency of your chiral separations. Additionally, scale up experiments will show how you can achieve high loadability while maintaining selectivity. Overall, new more robust chiral stationary phases provide enhanced capabilities over existing phases and this gives an opportunity to maximize the efficiency of chiral separations and purifications.

O#23

Title: Development of a 2D-Chiral HPLC/MS/MS Method for PF-03651639, its Enantiomer and a Metabolite

Authors: Y. Zhang
Institution: Pfizer Inc.
Contact: yizhong.zhang@pfizer.com

A chiral HPLC/MS/MS method for analyzing PF-03651639, PF-03651636 (enantiomers), and PF-03972563 (metabolite) has been developed. To minimize the potential impact of matrix effects, a 2D (two dimensional)-HPLC approach was applied. In this method, a protein precipitated plasma sample was first separated with a gradient program on a RP (reversed phase) HPLC column. Analytes were then "heart-cut" using a switching valve to a Chirobiotic TAG column (150 x 4.6 mm, 5 μm) running under isocratic conditions. The overall run time was 10 min. Matrix effects were assessed by comparing an extracted rat plasma sample and a monkey sample with a reference solution at the same concentration. No significant matrix effects were observed. Validation results indicate that the method is rugged and suitable to support GLP studies.

O#24

Title: A Screening Approach for Chiral Method Development Using Reversed Phase Gradient Elution and Immobilized Polysaccharide Stationary Phases

Authors: S. Babiak; H. Wang; R. Thompson; E. Loeser; P. Drumm
Institution: Chemical and Analytical Development (CHAD), Novartis Pharmaceuticals Corporation, East Hanover, NJ
Contact: stanislaw.babiak@novartis.com

A chiral screening protocol was implemented to enable *fast and simple* development of *reversed phase* methods for enantiomeric separation of *diverse* drugs and drug related compounds using *polysaccharide CSPs*. Chiral separations were initially performed in the isocratic mode. The protocol was improved by substitution of the multiple isocratic programs with a single shallow gradient, allowing for a dramatic decrease in the screening time. High success rate was achieved for functionally diverse test substances. In addition a smaller sample pool of Novartis compounds was used to validate this protocol.

O#25

Title: Enantiomeric Separation Using High Temperature Liquid Chromatography and Chiral Mobile Phase Additives

Authors: S. Ma, S. Shen, H. Lee, N. Yee, C. Senanayake, N. Grinberg
Institution: Boehringer Ingelheim Pharmaceuticals, In.c
Contact: shengli.ma@boehringer-ingelheim.com

Temperature as a valuable variable to control the performance of high performance liquid chromatography (HPLC) has attracted increasingly interests in both academic and pharmaceutical industry. We have applied HPLC under very high temperature (>90 ^{o}C) condition to perform chiral separation using pure water as mobile phase. By employing the sulfated -cyclodextrin (S- -CD) as chiral mobile phase additive (CMPA), baseline separation on a chiral amine molecule was achieved, for the first time. The effect of temperature, the concentration of S- -CD, and the interaction between the analyte and S-β-CD on the enantiomeric separation were evaluated and discussed.

O#26

Title: Enantiomer Recognition of Amino Acids with (+)18-Crown-6 using Reversed-Phase Liquid Chromatography and Nuclear Magnetic Resonance Spectroscopy

Authors: Z. Liu, R. Thompson, M. Lin, S. Shah, D. Drinkwater, P. Drumm
Institution: Novartis Pharmaceuticals
Contact: zhaoxia.liu@novartis.com

Enantiomers of primary amines were separated using a Chirosil RCA(+) stationary phase, derived from (+)-(18-crown-6)-2,3,11,12-tetra-carboxylic acid (18—C-6-TA), under reversed-phase liquid conditions. Retention and enantioselectivity were investigated with variation of aqueous and organic modifiers in the mobile phase. Nuclear magnetic resonance (NMR) experiments were designed under conditions that mimic the chromatographic conditions to further elucidate the nature of the interactions occurring between the probe molecule and the chiral stationary phase.

Session 5: LC-MS and Detection

O#27

Title: A Major New Application for Chromatography, Electrophoresis and Mass Spectrometry : Biosimilars

Authors: B. L. Karger
Institution: Barnett Institute, Northeastern University
Contact: b.karger@neu.edu

The generic version of small molecule drugs has been on the scene for 25 years, and, as we all know, has had a major impact on prescription medicines. Now generic versions of biologics will shortly be coming up for review with the regulatory agencies. However, biologics are up to 1000 times larger than small molecules and often have many post-translational modifications such as glycosylation. The likelihood of an identical version of the protein therapeutic to the innovator product is quite small. One of the critical steps in the comparison is the comprehensive characterization of the biopharmaceutical. This analysis is an area of importance for LC/MS and CE/MS and represents a major opportunity for new and more powerful technologies. In this talk, we shall overview the problem of comprehensive characterization of biosimilars, or biopharmaceuticals in general, and present approaches we have taken to advance the analysis of these compounds. We

will show separations of digested and intact proteins, the latter to characterize protein isoforms. The needs of these technologies in a regulatory environment will be discussed.

O#28

Title: Proteomics as a tool for optimization of human plasma protein separation and characterization of preparations of plasma-derived therapeutic proteins

Authors: D. Josic
Institution: Brown University and Rhode Island Hospital
Contact: djuro_josic@brown.edu

Human plasma is still a very important starting material for production of therapeutic proteins. However, many protocols for purification of plasma proteins were developed more than fifteen years ago, or even earlier. This time, the main concern for product development was the viral and clinical safety, and much less the purity of the therapeutic protein concentrate.
The application of proteomics technology in purification of proteins from human plasma and for characterization of plasma-derived therapeutics has been recently discussed. However, until now, the impact of this technology on the plasma protein fractionation and analysis of the final product has not been realized.
In the present work, we demonstrate the use of proteomic techniques the monitoring of different chromatographic steps as integral parts of the plasma fractionation.
In first step, anion-exchange chromatography, after the removal of immunoglobulin G (IgG) and non-binding proteins in the flow-through fraction, albumin and weakly bound proteins were eluted with low concentration of sodium chloride. The proteins that strongly bind to the anion-exchange column were eluted by higher salt concentrations. The non-binding proteins, mainly IgG, can be further fractionated by use of cation-exchange chromatography. In the last step, affinity- or immunoaffinity chromatography can be used for isolation of target proteins. The development of new chromatographic supports with the capacity up to one order of magnitude higher than the supports actually used opens new perspectives for use of chromatography, even for isolation of high abundant plasma protein such as IgG and human serum albumin.
The fractions of interest were analyzed, and proteins were identified by LC-ESI-MS/MS. By use of this method, not only the candidates for therapeutic proteins, but also some potentially harmful components could be identified.
In the last step, the final preparation of the purified therapeutic protein was characterized. For determination of batch-to-batch variations and differences between particular products, iTRAQ labeling technique was applied.
This strategy was very helpful for further process optimization, fast identification of target proteins with relatively low abundance, and for the design of subsequent steps in their removal or purification.

O#29

Title: Mass Defect: The Power of Separating peptide fragment ions in a mass spectrum

Authors: Y. Shi; X. Yao
Institution: Department of Chemistry, University of Connecticut
Contact: yu.shi@uconn.edu

The mass of a peptide differs characteristically from its nominal mass, which is equivalent to the number of protons and neutrons, by a value called the mass defect. Composed of a limited number of amino acids and a naturally-occurring variety of sequences, peptides have a restricted range of mass defect. Therefore, in a tandem mass spectrum, peptide fragment ions cluster discretely in each mass unit, leaving empty space not occupied by any native peptide fragment ions; this is the so-called forbidden zone. In this work, novel chemical methods are developed to generate ions of interest with large mass defect, including positive mass defect marker ions for phosphopeptides and peptide sequence ions that bear fragment ion mass defect labeling (FIMDL) tags. These ions, carrying the protein phosphorylation, quantitation or sequence information, are located in the unoccupied mass region in a spectrum and well separated from other interfering native peptide fragment ions.

O#30

Title: ELECTROCHEMICAL DETECTION IN BIOANALYSIS: Carbohydrates and Beyond

Authors: W. R. LaCourse
Institution: University of Maryland, Baltimore County
Contact: lacourse@umbc.edu

The growing areas of biotechnology and biopharmaceuticals have bolstered the need for bioanalytical methods with sensitive detection systems. The simple, sensitive, and direct detection of numerous polar aliphatic compounds is achieved with Pulsed Electrochemical Detection (PED). This technique exploits the electrocatalytic activity of noble metal electrode surfaces to oxidize various polar functional groups. In PED, multi-step potential-time waveforms at Au and Pt electrodes realize amperometric/coulometric detection while maintaining uniform and reproducible electrode activity. The response mechanisms in PED are dominated by the surface properties of the electrodes, and, as a consequence, members of each chemical class of compounds produce virtually identical voltammetric responses. Thus, PED *requires a priori separation* of complex mixtures via chromatographic or electrophoretic means. PED, which includes Pulsed Amperometric Detection (PAD) and Integrated Pulsed Amperometric Detection (IPAD), has gained prominence for the detection of carbohydrates.

Although PED is well-suited to the determination of carbohydrates, PED is also applicable to amine- and sulfur-containing compounds. The principles of PED are reviewed on the basis of voltammetric response of various functional groups. Detection strategies, optimization, and quantitation are discussed for particular classes of compounds.

Applications from excipients to active ingredients are included to highlight specific attributes and advantages of PED. Our discussion will include a look at an improved European Pharmacopoeia method for the determination of aminoglycosidic antibiotics; quantitation of drug glucuronide metabolites in urine, which is of great importance in interpretive forensic and clinical toxicology; and the use of novel sampling approaches (e.g., microdialysis) to monitor dynamic systems.

Csaba Horváth Lecture

O#31

Title: Performance of HPLC columns packed with Very Fine Particles. Consequences of the heat effect

Authors: F. Gritti, K. Kaczmarski, G. Guiochon
Institution: Department of Chemistry, The University of Tennessee, Knoxville, TN, 37996-1600, USA
Contact: guiochon@ion.chem.utk.edu

The demand from the pharmaceutical industry to increase speed, throughput, and resolution of HPLC analyses has pushed the manufacturers of packing materials to prepare new brands of fine silica particles. Several types of columns packed with sub-2μm particles are now commercially available. The permeability of these columns is much lower than that of conventional columns and their optimum velocity larger. So, in order to exploit their full potential, they must be operated at inlet pressures as high as 1 kbar. However, high linear velocities of the mobile phase combined with steep pressure drops along the column generate an important amount of heat, due to the friction of the mobile phase against the bed through which it percolates. This heat escapes through axial convection, radial and axial conduction. As a consequence, columns packed with very fine particles cannot be isothermal. Depending on the external environment (adiabatic column, moderately insulated column, or temperature-controlled column wall), significant longitudinal and/or radial temperature gradients are formed inside the column. This new type of column heterogeneity causes most serious problems that need to be investigated from both theoretical and experimental viewpoints. We measured and calculated the temperature distributions along and across 2.1 x 50, 100, and 150 mm columns packed with 1.7μm bridged ethylsiloxane - silica hybrid particles. We determined the relationship between their apparent efficiency and the mobile phase velocity. Emphasis will be given to the worst set of experimental conditions, in which the column wall is kept at a constant temperature, resulting in an important radial temperature gradient, hence in a large radial gradient of mobile phase velocity across the column. This gradient warps the solute bands and deleteriously affects the elution profiles of their elution peaks, hence the apparent column efficiency. We show the results of measurements of the temperature profiles along the wall of a column and across its exit section and illustrate the experimental dependence on the flow rate of the HETP of naphtha[2,3-a]pyrene. We compare calculated and measured elution peak profiles and calculated and experimental HETP curves for this compound. Finally, advice will be presented regarding the most appropriate ways to use this type of columns and conclusions drawn sketching the probable frontier of future applications of columns packed with ultra-fine particles.

LC-MS and Detection (Continues)

O#32

Title: Development and Validation of HPLC Stability Indicating Methods Using Charged Aerosol Detection Technology

Authors: M. Swartz; M. Emanuele
Institution: Synomics Pharma Services
Contact: mswartz@synomicspharma.com

A stability indicating method (SIM) is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. A SIM is a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. It is recommended that forced degradation or chemical stress studies be carried out to determine if analytical methods are stability indicating prior to embarking on long term stability studies. SIM's are routinely developed by stressing the API under conditions exceeding those normally used for accelerated stability testing. In addition to demonstrating specificity in SIM's, chemical stress testing, also referred to as forced degradation, can also be used to provide information about degradation pathways and products that could form during storage, and help facilitate formulation development, manufacturing and packaging. Stressing the API in both solutions and in solid state form generates the sample that contains the products most likely to form under most realistic storage conditions, that is in turn used to develop the SIM. In simplest terms, the goal of the SIM is to baseline resolve all of the resulting products (the API and all the degradation products) each from the other (i.e. no co-elutions). HPLC is a commonly used technique for SIMs, primarily with UV detection. However, not all degradants have UV chromophores, and therefore alternative detection technology must be investigated.

This presentation will discuss the development and validation of HPLC SIM methods and how utilizing a corona charged aerosol detector (CAD) in combination with a UV detector can provide distinct advantages. In addition to summarizing the utilization of CAD detector technology, we will provide examples of the analysis of compounds that so not possess chromophores sufficient for adequate SIM determination of the API or its degradants by UV detection alone.

O#33

Title: Genotoxic Impurity Control in Drug Substance Development

Authors: H. Lee; S. Shen; S. Ma; N. Grinberg
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.
Contact: heewon.lee@boehringer-ingelheim.com

Impurity profiling during drug substance development is critical for its safety, efficacy, purity, stability and quality. ICH guideline on "Impurities in New Drug Substance" specifies that impurities with unusual toxicities should be controlled with lower threshold. Genotoxic impurities pose significant analytical challenges due to its low level control required by regulatory guidelines. In this presentation, case studies of identification and control of genotoxic impurities are reported. The strategy to select the control point(s) is discussed as well. Depending on the nature of the compounds to be controlled, different chromatographic separation (HPLC and GC) methodologies are coupled with various detection techniques such as FID, UV and MS to support successful development of active pharmaceutical ingredient (API).

O#34

Title: Development of an HPLC-MS Method for Tracking Specific LBPA Variants in Biological Matrices in Response to Drug-Induced Phospholipidosis

Authors: V. V. Papov Jr; K. N. Locke; A. M. Mineo; B. Johnson; Z. Huang; J. H. Stoltz; S. Jayadev; J. A. Phillips
Institution: Boehringer Ingelheim Pharmaceuticals
Contact: vladimir.papov@boehringer-ingelheim.com

Phospholipidosis (PLD), which is characterized by the intracellular accumulation of phospholipids, can occur after dosing with a class of drugs known as cationic amphiphilic drugs (CADs). To mitigate the risk of induction of any potential accompanying toxicities associated with PLD it would be helpful to have a specific and sensitive biomarker for drug-induced PLD. Previously published reports have described lyso-bis-phosphatidic acid (LBPA), also known as bis(monoglycero)phosphate (BMP), as a potential biomarker for the onset and reversal of PLD. Consequently, a new LC-MS method has been developed utilizing multiple reaction monitoring (MRM) and a triple stage quadrupole mass spectrometer to specifically quantitate LBPA variants in biological matrices. For the initial studies, Male Hans Wister rats were dosed with an experimental phospholipidogenic compound (300 mg/kg daily) known to induce PLD at this dose level in rats. A separate group of rats were dosed with vehicle without compound as a control. In a parallel experiment, human monocytic U937 cells were incubated with varying concentrations of amiodarone, a known phospholipidogenic CAD. For both rat urine and U937 cells, the LBPA was extracted from the biological matrix using an organic solvent (3:1 chloroform:methanol (v/v)) followed by volume reduction and resuspension in a mass spectrometry compatible solvent. Two different LBPA variants, an asymmetric C34:1 (C16 and C18 chains with one double bond on the latter chain) and the symmetric C44:12 LBPA species were analyzed simultaneously using the LC-MS MRM method. Relative quantitation was accomplished by comparing LBPA peak areas from dosed animals with control animals. While C44:12 LBPA levels increased with increasing duration of exposure in the urine of rats dosed with the phopholipidogenic compound, the change in C34:1 levels was determined to be statistically insignificant. Conversely, the C34:1 LBPA increased with amiodarone concentration in U937 cells whereas the C44:12 showed statistically no change in these cells. These initial findings prompted a new study using Sprague Dawley rats and amiodarone dosing at 15 mg/kg/day (low-dose) or 150 mg/kg/day (high-dose) for 14 days. The amiodarone dosed rats were compared to control rats dosed at the same time with vehicle only. Urine for measurement of LBPA was collected daily with a subset of control and high-dose rats maintained for a 12 day recovery period. The sample preparation methodology for the LC-MS assay was refined to reduce sample handling and also lower the risk of LBPA degradation. In addition, C20:4 phosphatidyl glycerol was added to the method for use as an internal standard. LBPA changes were evaluated and compared to histopathology from exposure to amiodarone. Control and low-dose animals showed no significant changes in urinary C44:12 LBPA levels as compared to pretest values which was in line with microscopic analysis of the lungs, liver and leukocytes showing no evidence of PLD in the control animals although one animal in the low-dose group exhibited minimal PLD in the lung macrophages. In contrast, high-dose group urinary C44:12 LBPA levels increased steadily from drug day (DD) 2 to DD7, representing an approximately 10-fold change above corresponding pretest levels. Foamy alveolar macrophages characteristic of PLD were observed microscopically in the lung in 5/5 high-dose animals on DD14. Furthermore, there was a precipitous decrease in urinary C44:12 LBPA (high-dose group) after discontinuation of amiodarone dosing on day 14. Decreased C44:12 LBPA levels in recovery paralleled the absence or marked depletion of foamy alveolar macrophages in the lungs. Collectively, these data suggest that specific analysis of the C44:12 LBPA variant by MRM LC-MS could serve as a correlative urinary marker of drug-induced PLD.

POSTER ABSTRACTS

P#1

Title: Analysis of Absinthe by Solid Phase Extraction and LC-M/MS: A simple test for thujone concentration

Authors: J. Hackett; M. J. Telepchak; M. J. Coyer
Institution: Northern Tier Research, UCT Inc.,

AIMS:
Absinthe, once a beverage subject to a ban in the United States is now finding a new audience. It is euphemistically called the "Green Fairy" after the color of the liquid. The main constituent of the drink is Thujone, which is thought to give rise to its hallucinogenic properties. The drink is also known to contain Anethole and upto 50 percent acohol by volume. The focus of this presentation is to to demonstrate a simple and efficient procedure for the analysis of Absinthe which will determine the presence (or absence) of thujone as well as the anethole. This should be of great assistance to those actively testing such drinks and offer an alternative method of analysis.

METHODS:
Genuine samples of Absinthe (9), calibrators and controls were used for extracting anthethole and thujone from 50 PC aqueous alcohol samples (0.2 mL) containing menthol as an internal standard. In this method, the samples were diliuted with DI water and applied to solid phase extraction columns (3 mL containg 25 mg of CEC30 sorbent (UCT Inc.,). The columns were conditioned with methanol and DI water (1 mL, respectively). After washing with DI water the samples were eluted with 4 x 50 µL of methanol and the eluates transferred directly to auto sample vials for analysis by LC-MS/MS in positive electrospray (MRM) mode.
From the analysis of the calbrators and controls: r^2 value> 0.995, recoveries > 90%, and a limit of detection of 10 µg/ mL, respectively were acheived. The method was found to be linear upto 200 µg.
At NTR, an API 2000 MS/MS instrument was employed with a 50 x 2.1mm (3 µm) Phenyl column (Selectra ™) for separation of the analytes. A mobile phase consisting of acetonitrile (with 0.1% formic acid) and DI water (with 0.1% formic acid) was used in gradient mode in the analysis.

RESULTS:
Data is presented in this poster along with LC-MS/MS chromatograms showing those samples of genuine absinthe containing thujone and those that containing only anethole. The range of thujone concentrations was found to be from 0 to over 100 µg whilst all of the samples contained Anethole at various levels far exceeding that of the thujone.

CONCLUSIONS:
This simple amd effiecient procedure for the analysis of Absinthe (especially Thujone) is the first method using both SPE and LC-MS/MS. The use of this procedure should assist those analysts involved testing Absinthe beverages for the presence of thujone. This method should also help analysts testing pre-ban samples of Absinthe for authenticity, as the presence or absence of the thujone should assist in the establishment of legitimacy of the sample.

P#2

Title: Selective Depletion of Phospholipid Interference Utilizing HybridSPE-PPT Technology

Authors: C. R. Aurand
Institution: Supelco

Analysis of biological samples is often hindered due to interferences carried through the sample preparation technique. Protein precipitation is a widely accepted sample preparation method for biological plasma samples due to simplicity and gross level removal of proteins. Though widely used, protein precipitation methods often result in chromatographic irregularities due to co-extracted endogenous species such as phospholipids that negatively affect chromatographic analysis. A more thorough sample clean up can be achieved using solid phase extraction (SPE), but at a cost of time and method complexity.

In this presentation a new platform was developed to process various plasma samples using a simplified two-step procedure to produce biological samples depleted of phospholipids prior to LC-MS-MS analysis. The HybridSPE-PPT platform employs the simplicity of standard protein precipitation with the added selectivity of SPE. The platform exhibits a high affinity towards phospholipids while remaining non-selective towards a broad range of basic, neutral and acidic compounds.

P#3

Title: Evaluation of Operating Conditions in Analytical and Preparative SFC

Authors: L. Miller
Institution: Amgen

Over the past years supercritical fluid chromatography (SFC) has become an established process for analysis and purification in the pharmaceutical industry. The majority of these applications are for chiral separations with an increasing number of achiral separations being reported. As SFC is a younger technology compared to HPLC, many of the theoretical studies that have been performed for liquid chromatography have not been performed for supercritical fluid chromatography. Over the past year Amgen has performed a number of research projects to investigate operating conditions for analytical and preparative SFC.

This talk will discuss the results of these research projects. Topics to be discussed include 1) evaluation of co-solvents for chiral analytical SFC separations, 2) injection techniques in preparative SFC and 3) effect of co-solvents on preparative SFC separations

P#4

Title: Ion Mobility Spectrometry --- Applications in Cleaning Verification and Potential for Pharmaceutical Analysis

Authors: C. Qin, A. Granger, J. McCaffrey, D.L. Norwood
Institution: Boehringer-Ingelheim Pharmaceuticals, Inc.

Ion mobility spectrometry (IMS) is an analytical technique which separates ions based on their gas phase ion mobility. It is able to routinely achieve high sensitivity, high specificity and high selectivity with high speed data acquisition. Using IMS, a single analysis can be completed within a minute. The features of IMS provide great potential for pharmaceutical analysis. In this presentation, several case studies using IMS technology for cleaning verification are reported. The cleaning verification methods were validated with respect to specificity, linearity, precision, accuracy, and quantitation limit. The second application of IMS in pharmaceutical industry is in the worker exposure safety study area. An IMS method was developed and validated for analysis of a drug substance in glass fiber filters in support of a thirteen-week inhalation toxicity study. The results obtained from IMS method were comparable with those from HPLC_UV method. The total time of analysis was reduced significantly compared to HPLC analysis. Other potential pharmaceutical applications of IMS such as leachables and extractable analysis, positive control of clinical supply analysis, and content uniformity test will also be discussed.

P#5

Title: Comparison of Chiral Separations Using High Performance Liquid Chromatography and Supercritical Fluid Chromatography on Coated and Immobilized Amylose and Cellulose 3,5-Dimethylphenyl Carbamate Columns

Authors: K. A. Nadeau
Institution: Amgen

Within the pharmaceutical industry, two coated polysaccharide-based stationary phase columns are commonly used for chiral analyses, amylose 3, 5-dimethylphenyl carbamate (AD-H) and cellulose 3, 5-dimethylphenyl carbamate (OD-H). Limitations of these columns include incompatibility with solvents (e.g., tetrahydrofuran, methyl tert-butyl ether, dichloromethane, and ethyl acetate) and the inability to withstand high pressure. Recently, a more robust line of columns, immobilized amylose and cellulose 3, 5-dimethylphenyl carbamate stationary phases (IA and IB respectively), was introduced to allow greater freedom in solvent choices. Additionally, another coated amylose and cellulose 3, 5-dimethylphenyl carbamate stationary phase (Amycoat and Cellucoat respectively) with 3 and 5 μm particles has been introduced that allows chromatography at higher pressures.

This research compiles the comparison of commercially available chiral separation columns using normal phase high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). In particular, the chromatographic performance of the established coated phases (AD-H and OD-H) is compared with the corresponding immobilized columns (IA and IB) and the newly introduced coated phases (Amycoat and Cellucoat). The separation capabilities of these columns were probed with acidic, basic and neutral compounds. Furthermore, since both HPLC and SFC are widely used for chiral separations, the consideration of column performance using both techniques was evaluated as the stationary phases, column dimensions, sample diluent, modifiers and additives were kept constant.

P#6

Title: Cationic-Protein Binding to Vesicles: Asymmetric Location of Anionic Phospholipids

Authors: F. Torrens; G. Castellano
Institution: Universitat de València

The role of electrostatics is studied in the adsorption of cationic proteins to anionic (phosphatidylcholine / phosphatidylglycerol, PC/PG) and zwitterionic (PC) small unilamellar vesicles (SUVs). For model proteins the interaction is monitored *vs*. PG content at low ionic strength. The adsorption of lysozyme and myoglobin (isoelectronic point, p*I* 7–11) is investigated in SUVs, along with changes of the fluorescence emission spectra of the cationic proteins, *via* their adsorption on SUVs. In the Gouy–Chapman formalism, the activity coefficient goes with the square of the charge number. Deviations, from the ideal model, show the asymmetric location of the anionic phospholipid in the bilayer inner leaflet, in mixed zwitterionic/anionic SUVs for both lysozyme– and myoglobin–PC/PG systems, which is in agreement with experiments and molecular dynamics simulations. The effective SUV charge stays constant. The effective – formal difference increases 0.417 e.u. The effective protein charge increases as PC/PG < PC being greater for myoglobin. The molar free energies of the protein in aqueous and lipid phases increase as PC < PC/PG. Both free-energy changes are greater for myoglobin. The effective interfacial charge stays constant for anionic PC/PG SUVs being greater for myoglobin. Provisional conclusions follow. (1) With the Gouy–Chapman formalism is obtained as ln $\propto$ $\cdot\sinh^{-1}($ $) \approx$ ^{2}. The activity coefficient goes with the square of charge number. As $\propto$ at constant it can be expected ln $\propto$ ln $\propto z_L\cdot\sinh^{-1}(z_L) \approx z_L^2$. For the lysozyme – and myoglobin–PC/PG systems in mixed zwitterionic/anionic vesicles, deviations from the ideal model show the asymmetric location of the anionic phospholipid, in the inner leaflet of the bilayer, in agreement with experiments and molecular dynamics simulations. The behavior of the PC molecules on both leaflets is expected to behave almost independently of each other; each leaflet seems to follow an independent behavior, in that the changes in one leaflet did not correlate with the lipid behavior of the other. (2) The effective vesicle charge stays constant and null for zwitterionic PC vesicles. The effective – formal difference increases 0.417 e.u. because of high surface coverages. The effective protein charge increases as PC/PG < PC because PG provides greater screening. The change caused by PG content is greater for myoglobin because of its greater physical charge. (3) The molar free energies of the proteins, in aqueous and lipid phases, increase as PC < PC/PG because PG decreases protein charge, destabilizing protein–water and –vesicle electrostatic interactions responsible for protein solvation. Both free-energy increments caused by PG are greater for myoglobin, because of its greater physical charge. (4) The effective interfacial charge is a determinant factor to obtain a unique value of the binding constant independently of the surface charge density of the vesicles. The effective interfacial charge stays almost constant for anionic PC/PG vesicles, because PG does not change effective vesicle charge. The charge is greater for myoglobin because of its greater physical charge.

P#7

Title: Automated Approach to Eliminating the Concentration and Dry Down Bottleneck Associated with Purified Compounds from Preparative Chromatography

Authors: J. Stevens, Ph.D; L. Roenneburg
Institution: Gilson, Inc.

Preparative chromatography is the designated purification technique employed for pharmaceutical, nutraceuticals and biologically compounds, showing possible activity. It is imperative that a quantity of purified compound be available for activity assays, additional synthesis and storage. Researchers work extremely hard to create an active compound; however there is always an impurity or non-reactive component that the FDA requires to be isolated, removed or defined. Studies have shown that synthetic scale up can create impurities or additional bi-products that were not observed at an analytical scale compromising the purity of the active compound. Therefore preparative chromatography is employed to separate and isolate the active compound. On average when semi-preparative chromatography is employed (e.g. 21.1 x 50 mm, column geometry, flow rate 15-50 mL/min, compound mass 5-300 mg) the average volume associated with the purified fraction is approximately 5-10 mL. Scaling the purification to a preparative level (e.g. > 50 x 150 mm, column geometry, flow rate > 75 mL/min, compound mass 0.3 – 15 gm) creates a fraction volume 2 to 10 times the fraction volume stated above (e.g. 20-120 mL). The purified fraction collected requires obviously dry down to remove the mobile phase. Since the common preparative technique employed for purification is RP HPLC the purified fraction will contain an aqueous and organic component including a modifier (e.g. TFA, TEA, formic acid). Elimination of the solvent associated with the purified fraction requires a vacuum and a temperature increase. Concentration of the mobile phase modifier has been shown to cause hydrolysis of functional groups, possibly destroying the activity of the purified compound. In addition evaporation or dry-down is time consuming (e.g. 8-24 hrs) and requires manual intervention. Employing an activated media packed in a SPE cartridge the automated system can intelligently identify the purified fractions, and concentrate the purified compound onto the SPE cartridge, eliminating all the possible caveats and limitations regarding preparative purification. This approach allows over 30 purified fractions/compounds to be processed in less than 2 hours without manual intervention. General cost savings is approximately $5,000/week based on research/instrumentation reduction.

P#8
Title: A Totally Automated System for Purifying Large Quantities of Sample via Shallow Isolation Gradients or Stacked Injections and Fraction Collection
Authors: J. Stevens, Ph.D; L. Roenneburg
Institution: Gilson, Inc.

Preparative chromatography is often employed to purify large quantities of compounds. This usually involves a large internal diameter column in the 5 cm or more range. These preparative columns are very expensive and have bed instabilities associated with their use. Because of the cost associated with synthesizing large (gram) quantities of a compound much time and labor is put forth prior to the preparative synthesis to optimize the synthesis and minimize any and all byproducts that will decrease the overall recovery of the compound of interest. Many times the synthesis is very successful and only a small amount 2-5% of the overall mass is associated to a byproduct. However this is still a byproduct and needs to be separated from the major component. Two avenues being explored by many chromatographers is to implement continuous injections to purify the compound on smaller preparative columns. Continuous injection “stacked injection” of sample one right after another under isocratic conditions with continuous fraction collection allows for an increase of throughput greater than 50% for both the number of samples and amount (gram quantities) of sample purified. This saves a significant amount of time since the system is not waiting for the fractions to be collected before beginning the next injection. Being able to collect onto multiple fraction collector beds also increase its efficiency and usefulness. On the other hand when the synthesis produces a complicated chromatogram where isocratic conditions are not applicable “shallow isolation gradients” allows for a very simple approach to compound purification. In which the retention time of the compound of interest is implemented into the shallow gradient equation to determine the initial and end mobile phase composition. Both these techniques are based on an analytical scout analysis which determines which techniques should be used. Upon the analytical determination the system automatically continues in a semi-preparative purification scheme. The data presented for both techniques offers a surprisingly simple approach to the purification of large quantities of compounds with continuous fraction collection.

P#9
Title: Increasing Throughput of Gel Permeation Chromatography (GPC) Clean-up via a Parallel Sample Extraction Process, Collection Window Determination, and Individual Collection per Column
Authors: J. Stevens, Ph.D; M. Halvorson, Ph.D.
Institution: Gilson, Inc.

Gel Permeation Chromatography (GPC) is a highly effective and efficient post-extraction clean-up method for the removal of high molecular weight interferences such as lipids, proteins, humic acid and pigments from sample extracts prior to analysis. Post-extraction GPC clean-up is used extensively for preparing food and environmental samples (such as soils) prior to analysis for insecticides, fungicides, herbicides, semi-volatiles, PCBs and other environmental contaminants. GPC clean-up has many benefits, including decreased instrument maintenance costs, higher quality of analytical data, enhanced analytical column life and its automation capabilities. One of the disadvantages of GPC clean-up is the relatively slow throughput per sample extract compared to other sample clean-up methods. The purpose of this study was to evaluate the effectiveness of developing an automated GPC clean-up system that is capable of running 2-4 individual sample extracts in parallel, along with fraction collection per column, thus improving sample throughput. Data is presented using a USEPA GPC calibration standard to determine the time window for fraction collection. A very simple acquisition software program is used to determine this collection window. One established unique food/environmental samples can be injected simultaneously onto the individual stainless steel GPC columns with independent fraction collection per column.

P#10
Title: Method Development and Validation for the Determination of Gentamicin by HPLC
Authors: J. Cabinian, X. Wang , L. Alquier
Institution: Cordis Corporation

Gentamicin, an aminoglycoside antibiotic produced by the genus *Micromonospora*, has been in use for several decades particularly for gram-negative infections. An HPLC method was developed and validated to determine gentamicin’s major components in drug substance. Several challenges were encountered during method development and validation. First of all, gentamicin exists as a collection of four closely related molecular species: gentamicins C1, C1a, C2, and C2a. These compounds, and other related substances, are polar in nature and are readily soluble in aqueous media. It is a daunting task to retain these species in the column under reversed-phase conditions, to separate molecules from each other and from the other related molecules. Ion-pair agents (like TFA or PFPA) were tried and various columns were evaluated before optimal separation can be achieved. In addition, gentamicin components do not have UV-absorbing chromophores. A universal detection system, the Corona charged aerosol detector (CAD), was used in the method described here to measure the gentamicin components. The validation of the method, which will include the characteristics accuracy, precision, linearity, and limit of quantitation (LOQ), will be presented. Due to the presence of various components in the active pharmaceutical ingredient (API), the LOQ determination is different from that of a conventional single-peak API.

P#11

Title: Newly synthesized stationary phase used on method development for α- and β-emitters measurements in environmental samples

Authors: R. I. Olariu; C. Arsene; D. Humelnicu; M. Duncianu
Institution: "Al.I. Cuza" University of Iasi, Faculty of Chemistry

(Iso)polyoxometalates represent a wide class of molecular clusters with an almost unmatched range of physical properties and ability to form structures that can bridge several length scales [1]. Because of their structures and properties these isopolyoxometalates give rise to interest in interdisciplinary areas such as materials science catalysis, separation, imaging, and biological ion transport models [2-5]. The high thermal stability and radiation-resistant nature of (iso)polyoxotungstates in particular attract attention to the use of these species for the separation and sequestration of radioactive species [6-7].
An IC method for the determination of α- and β-emitters (UO_2^{2+} and Th^{4+}) has been proposed to be developed, using the newly synthesized isopolyoxometalates as stationary phase for the immobilization of radioactive ionic metals. In order to establish optimal separation condition the method development involves the study of acid media as pH modifier, temperature, amounts of chemical target, chemical interferences, and flow rates. At the optimal pH, where more than 80% of both UO_2^{2+} and Th^{4+} are retained from pure standard solution, the chemical interferences of several know metallic cations that occurs in most environmental samples (Al, Fe, Pb, Cr) have been investigated.
Examples of newly synthesized (iso)polioxomolybdate and (iso)polioxotungstate that incorporate the sterically demanding linear UO_2^{2+} and Th^{4+} ions are also reported. Both (iso)polioxomolybdate and (iso)polioxotungstate retained uranyl and thorium ions in aqueous system at pH = 4.5 for UO_2^{2+} and pH = 6.5 for Th^{4+} leading to the newly complex combinations of these cations in different molar ratios. Accomplishments for the chemical characterization of the newly synthesized units is foreseen through the data obtained by the mean of state of art technique as Fourier Transformed Infrared Spectroscopy (FTIR), UV-Vis spectrophotometry, scanning electronic microscopy (SEM) with electron diffraction X-ray (EDX) detection, and thermogravimetric analysis.

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P#12

Title: Fast reversed-phase ion-pair liquid chromatography in the simultaneous analysis of nitrite and nitrate in rainwater samples collected in the north-eastern Romania over 2003-2008

Authors: C. Arsene; R. I. Olariu; M. Ponzo; D. Vione
Institution: "Al.I. Cuza" University of Iasi, Faculty of Chemistry

Nitrate (NO_3^-) and nitrite (NO_2^-) are important components in atmospheric liquids and surface water where they play an important role in photochemical and photobiological processes concerning the transformation of dissolved molecules, plus the connected possible production of harmful compounds, and the penetration of radiation inside the water column.
Actually, nitrate (NO_3^-) and nitrite (NO_2^-) photolysis significantly enhances the photooxidation of natural and anthropogenic pollutants by formation of hydroxyl radicals (OH), one of the most powerful oxidant in aqueous solution. If nitrate has been routinely determined in many surface- ground- and rain-water surveys, for nitrite measurements in such samples are very scarce.
There is evidence that nitrite photochemistry in surface waters has often been underestimated because of ion chromatography, the standard technique adopted for anion (and therefore nitrate) quantification in such matrices but which is poorly sensitive for nitrite (which would often be below detection limit although its photochemistry could still be very significant). Accordingly, the assessment of nitrite photochemistry in surface water samples requires more sensitive, dedicated analytical techniques.
Nitrite is well determined by reaction of derivatization with N-(1-naphthyl)ethylenediamine to origin a colored product (Griess reaction) detected through spectrophotometric method. In order to achieve a simultaneous determination of nitrite and nitrate, several methods have been also developed such as flow injection analysis (FIA) with spectrophotometric determination, ionic chromatography (IC) with suppressed conductivity detection or, more recently, capillary electrophoresis (CE). However, among them, some problems have been reported such as incomplete reduction and sample carryover (FIA) or relatively long analysis time required (IC).
In the present work an ion-pair HPLC instrumental analytical method has been used for the simultaneously analysis of nitrate and nitrate in 200 rainwater samples collected in the north-eastern Romania over 2003-2008 period. A flow rate of 1 mL min^{-1} and a mobile phase made up of 83% tetrabuthyl-ammonium bromide 3.0 mM (TBA-Br titrant) in a 50.0 mM sodium phosphate buffer at pH 3.0 and 17% acetonitrile organic solvent, result in a fast, accurate and efficient separation of both nitrate and nitrite in the analyzed rain-water samples. The method was optimized by looking on several mobile

phase variables, including the acetonitrile ratio (from 30% to 10%) and other quaternary salts apart the brominated one, pH of the mobile phase (from 3 to 6) and mobile phase flow rate (from 0.2 to 1.4 mL/min).
For nitrate, the results obtained via ion-pair liquid chromatography are in very good agreement with those obtained by ion chromatography (slope of 1.04±0.09 and Person coefficient of 0.99).
The obtained results prove that the ion-pair liquid chromatography and reversed-phase chromatography is advantageous about the cost (instrumentation and columns), but also concerning a considerable number of parameters (stationary phase material, counter-ion and concentration, pH and ionic strength of eluent) for the simultaneously determination of both nitrate and nitrite ions from natural water samples.

P#13

Title: Investigation of Chiral Separation by GC using Chirasil-Val as Stationary Phase

Authors: Q. Chen; N. Grinberg; S. Ma; J. Lorenz; S. Kapadia; Y. Wang; C. Manolescu; H. Lee; S. Shen; J. McCaffrey; D. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.

Chiral separation remains a challenge due to a poor understanding of the interrelation between the enantiomeric analytes and the chiral stationary phases. A better understanding of such relationship will reduce the challenge and improve the productivity. Chiral separation using gas chromatography is less challenging since the mobile phase has little or no interaction with either the analytes or stationary phase. Chirasil-Val is one of the commercially available diamide stationary phases and contains two hydrogen-bonding sites. Numerous applications of Chirasil-Val in chiral separation have demonstrated the significance of hydrogen bonding for its chiral selectivity. In this poster the chiral separation of a series of 1, 2,3,5-tetrasubstituted 4- imidazolidinone compounds on Chirasil-Val was studied. The influence of structural differences on the separation was discussed. The retention behavior and the resolution of these compounds were compared to understand the chiral recognition mechanism.

P#14

Title: Use of 2-mm I.D. C18 Monolithic Chromatography in a Discovery Setting: A Means for the 3X-Reduction of Cycle Times

Authors: J. M. Hollembaek, B. J. Rago, A.King-Ahmad, K. F. Wilkinson, C.L. Holliman
Institution: Pfizer Global R & D

Programs that are in the discovery phase of pharmaceutical development typically generate only one or two sample sets per compound before the compound is discarded for a more desirable molecule. Therefore LC/MS/MS methods that analyze these sample sets are developed quickly using rapid gradients and more rigorous assay development is reserved for compounds that are deemed favorable and will have a longer lifecycle. Although initial assays are relatively generic, chromatographic selectivity is important because the samples are in complex matrices and may contain unknown metabolites and dosing formulation components. Therefore bioanalytical chemists that develop assays for discovery compounds must compromise on selectivity, which typically increases runtimes, with throughput which is needed to support multiple studies with minimal resources and costs.

We have found that the introduction of 2 mm ID monolithic columns have provided us with means to improve both the selectivity and reduce costs of our discovery assays, by allowing higher flow rates while providing peak efficiency near that of a 3 μm particle column in a 50x2mm format with system pressure well within the capabilities of conventional HPLC hardware, while the added flow rate reduces the cycle time per injection two thirds, leading to higher throughput on a daily basis, reducing our cost per study. The monolithic column is a porous silica rod, biporous structure which consists of micrometer-size through-pores and meso- or microporous silica skeleton. C18 is bonded in situ and a PEEK coating is applied to the outer surface. The C18 bonded silica monolithic HPLC column has been compared with the efficiency 3 μm packed column with the permeability of a 15 μm packed column.

Here we provide a comparison of separations on monolithic and particle base columns along with concentration data comparisons, void volume chromatograms, regions of phospholipids retention chromatograms.

In our Discovery Pharmacokinetics Bioanalytical laboratory, we have found the 2-mm I.D. C18 Monolithic, gave adequate selectivity in a 0.5 minute chromatogram, with system pressure at 2500 psi, well with in the range of conventional HPLC equipment. At a flow of 1.6 ml/min, cycle times of 0.5 minutes are now possible. The major factor in achieving a cycle time of 0.5 min now becomes the cycle time of the HPLC and MS hardware. At a flow rate of 0.8 ml/min chromatograms of 1 minute with cycle time of 1.4 minutes and system pressure of 1200 psi are now routinely run in our laboratory. The 2-mm I.D. column has demonstrated exceptional ruggedness by use of one column in 57 studies with approximately 150 injections pre study with minimal change in separation and system pressure with compared with a new column.

P#15
Title: Effect of Ionic Liquid Anions on the Retention of Naphthalene Derivatives in SFC
Authors: Y. Wang; N. Grinberg; Q. Chen; C. Manolescu; J. McCaffrey; D. L. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals

Room temperature ionic liquids have been proven to be of great interest in many areas of analytical chemistry. They have been utilized as stationary phases in both GC and HPLC. Recently, we evaluated the ionic liquid stationary phases in Supercritical Fluid Chromatography (SFC). The results showed that ionic liquid significantly improved the separation efficiency of polar or charged compounds in SFC [1]. In this study, the effects of ionic liquid anions on the separation of naphthalene derivatives were investigated. Ten millimoles of 1-butyl 3-methyl imidazolium with different anions including chloride, phosphate, methanesulfonate, tetrafluoroborate, octylsulfate, and diethyleneglycolmonomethylethersulfate were coated on silica columns. Naphthalene derivatives including 1-methylnaphthalene, 1-nitronaphthalene, N,N- dimethyl -1-naphthamine, 1-hydroxynaphthalene, 1-naphthoic acid, 1-aminonaphthalene and 1-naphthalenemethylamine were used as analytes. The results show that the anions of ionic liquid also affect the separation of investigated analytes, especially for naphthalene with amine moieties. The mechanisms of the interaction between ionic liquid anions and the analytes are discussed. Comparisons of the stabilities of the ionic liquid columns with different anions are also presented.

[1] Yuwen Wang, Nelu Grinberg, Qian Chen, Cristina Manolescu, John McCaffrey and Daniel Norwood, Evaluation of Ionic Liquid as Stationary Phase in Supercritical Fluid Chromatography, 2008 *Eastern Analytical Symposium*, Garden State Convention Center, Somerset, New Jersey, Nov. 17-20, 2008.

P#16
Title: Research and Testing of Biodiesel, a State of Connecticut Alternate Energy Initiative
Authors: J. D. Stuart; R. S. Parnas; C. R. Perkins
Institution: University of Connecticut

To lessen its dependency on Middle East oil, The State of Connecticut's alternate energy initiative has sought to expand the use of biodiesel. Biodiesel (often referred to as B-100) when mixed with regular petroleum diesel in mixtures up to 20 per cent by volume (B-20) is used to directly power diesel vehicles and burned in commercial, home, school and State heating units. This presentation will detail how The University of Connecticut's Biofuel Consortium has responded to the State's initiative by performing research on the effective pretreatment of recycled waste cooking oil that can be used as the feedstock. We have developed both batch and continuous biodiesel reactors using energy-efficient methods to produce biodiesel. This presentation will detail how the final B-100 product is tested following a series of American Society of Testing Materials tests (ASTM D 6751-08). One of the most important of those tests is a high temperature, gas chromatographic method that in a 30-minute run, separates any free glycerol (a by-product) and various incompletely-transesterified mono-, di- and tri-glycerides, from the methyl esters (collectively referred to as biodiesel) (ASTM D 6584-08).

P#17
Title: UPLC and RRLC with the Corona ultraTM Charged Aerosol Detector
Authors: M. Plante; C. Crafts, B. Bailey; I. Acworth
Institution: ESA Biosciences

The use of the Corona® Charged Aerosol Detector (CAD®) has become standard for many applications. New technology of Rapid Resolution Liquid Chromatography (RRLC) and Ultra-High Pressure Liquid Chromatography (UHPLC) has allowed for a faster peaks and shorter methods. Universal detection of these peaks with minimal peak broadening is required to maximize the effectiveness of this new technology. ESA Biosciences has introduced the next generation of Charged Aerosol Detection with the Corona *ultra*. This detector incorporates the features introduced in 2004 with Corona CAD and now include faster response times and filter technology, which allows new compatibility with conditions from UHPLC to normal HPLC. Peak widths as small as one second have been analyzed. The incorporation of new controls and prompts also make monitoring of the detector's performance and required maintenance worry free and care free.

P#18
Title: Electrochemistry On-line with LC-MS to Produce and Characterize Drug Metabolites and Degradants
Authors: I. Acworth
Institution: ESA Biosciences

The integration of predictive metabolic studies into early, high-throughput phases of drug discovery is of critical importance toward reducing attrition rates in later stages of drug development.

Phase I drug metabolism frequently involves oxidative or reductive (redox) biotransformation reactions. By using coulometric electrochemical (EC) cells online prior to mass spectrometry (MS) we have previously demonstrated, in agreement with literature, that it is possible to electrochemically mimic certain biological Phase I redox reactions (e.g. N- and O-dealkylation, N-, S-, C- oxidation and dehydrogenation reactions). The hyphenated term 'electrochemistry – mass

spectrometry' (EC-MS) used herein refers to the measurement of both charge transfer (via EC detector) and nature of products formed (via MS) from EC oxidation of 'substrate' as a function of EC cell potential.

The objective of these studies was to more closely examine the association between EC oxidation and a) in vitro biological metabolic stability and b) product /metabolic profiles. Compounds were:

• Directly analyzed by flow-injection analysis (FIA) with on-line coulometric EC reaction (oxidation) prior to detection with electrospray ionization single quadrupole MS.

• Incubated with pooled human hepatic microsomes and analyzed by fast gradient HPLC- coulometric array detection.

• Analyzed by pre-column on-line coulometric EC reaction prior to fast gradient HPLC- coulometric array detection.

Data on EC potential dependence of oxidative consumption of substrate (EC-MS), the profile of resulting products and their comparison to in vitro biological metabolic experiments are discussed.

P#19

Title: AFFF/MALS and Ion Mobility for Analysis of Antibodies and Non-Covalent Complexes

Authors: B. A. Andrien, Jr.
Institution: Alexion Pharmaceuticals

Characterizing large protein therapeutics and macromolecular complexes over 200kDa is an analytical challenge. Techniques that can be used include SEC, SDS-Page, LALS, RALS, DLS, AUC, etc. some of which have a low upper mass limit. Two techniques that we have used in our lab are Asymmetric-Field-Flow-Fractionation Multi-Angle Light Scattering (AFFF/MALS) and Ion Mobility Spectrometry (IMS) to analyze different protein mixtures including: monoclonal IgG's, IgA, IgM, Keyhole Limpet Hemocyanin (KLH), aggregates, and non-covalent complexes.

AFFF is a separation system based on liquid phase diffusion in a cross flow. AFFF coupled with MALS gives an orthogonal determination of the average liquid phase molecular weight/radius and works well for well separated homogeneous peaks. Using AFFF/MALS we were able to analyze the average liquid phase molecular weight/radius for protein mixtures in formulation or physiological compatible solvent systems with average run times of 30-60 minutes.

IMS is a separation system based on atmosphere gas phase ion mobility as a function of molecular radius. Using IMS alone we were able to get direct infusion gas phase molecular weight/radius profiles for protein mixtures in solvents that can be electrosprayed. The IMS mass spectra give good m/z detail with a resolving power of 5 and mass accuracy of 5-10% across a large mass range 10 kDa to 10 MDa with average run times of a few minutes.

We have coupled AFFF/MALS to IMS which gives both the liquid phase average mass and gas phase molecular weight profiles as a function of retention time in solvents that can be electrosprayed. AFFF/MALS is complemented with the mass spectra of IMS especially when the chromatography is unable to fully resolve the mixture components into homogeneous peaks. AFFF/MALS/IMS produces LC-MS like data for macromolecular over 200kDa with IMS scan times on the order of 20 seconds with AFFF/MALS elution peak widths greater than 1.5 minutes.

P#20

Title: HPLC Enantiomeric Separation of an Aromatic Amine using Crown Ether Tetracarboxylic

Authors: S. Shen; S. Ma; H. Lee; N. Yee; C. Senanayake; N. Grinberg
Institution: Boehringer-Ingelheim Pharmaceuticals, Inc.

Separation of an aromatic amine is presented using a commercially available stationary phase - Chirosil RCA (+) which consists of a chemically bonded crown ether tetracarboxylic acid. To separate this amine we used mobile phases containing organic mobile phase such as methanol or acetonitrile and 0.1% $HClO_4$ in water. Plots of ln k' vs. the volume fraction of the organic modifier as well plots of ln k' and vs. 1/T showed a non-linear behavior which prompted us to look at non chromatographic techniques. These results prompted us to undertake non chromatographic experiments, such as infrared spectrometry and vibrational circular dichroism, in order to elucidate the particular interactions between the chiral amine and the crown ether tetracarboxylic acid.

P#21

Title: Practical Application of Fused-Core® Columns with Standard HPLC Instrumentation: Understanding the Importance of Extracolumn Volume

Authors: T. J. Waeghe; R. T. Moody; C. L. Zimmerman
Institution: MAC-MOD Analytical

Since the introduction of columns packed with sub-two-micron particles for high-throughput and high-efficiency separations over five years ago, a number of HPLC practitioners have used them for improving productivity or for separating very challenging samples in their laboratories. However, the significantly higher pressures necessary to utilize fully such

columns require the purchase of instrumentation capable of delivering pressures of 600-1000 bar. Many chromatographers and laboratory managers have been reluctant to purchase new higher pressure instruments for their laboratories because of the costs involved, the lack of familiarity with the requirements and limitations of the new technologies, and most recently, the severe downturn in the economy. Fortunately, the innovative development and commercialization of columns packed with superficially porous, 2.7-micron HALO® Fused-Core® particles have now opened the arena of fast UHPLC and ultrahigh resolution separations to those laboratories having conventional 400-bar instrumentation.

To make it easier for those who want to use these fast, high-resolution columns effectively, we have evaluated experimentally the impact of extracolumn volume and dispersion on column efficiency for a number of commercially available Fused-Core column geometries in terms of (1) injection volume, (2) tubing volume, (3) flow cell volume, and (4) detector response time. We have summarized the results and recommendations in practical terms for the novice and experienced chromatographer, so that they can achieve optimum efficiency and performance. With this turn-key guidance, users of Fused-Core columns will be able to obtain "UPLC-like" performance at the moderate pressures within the reach of common 400-bar instruments.

P#22

Title: An HPLC Method Using Charged Aerosol Detection for the Quantitative Determination of Methylsulfonyl Piperidine Analyte Lacking a UV Chromophore

Authors: M. Jerfy; A. Soman
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.

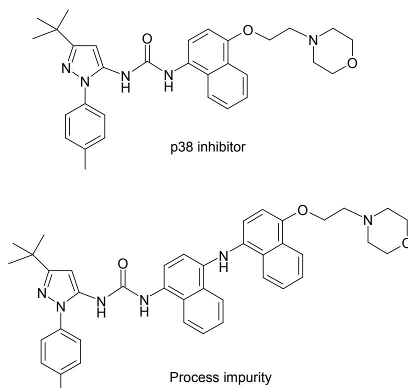
A simple isocratic reversed phase high-performance liquid chromatographic method (HPLC) with charged aerosol detection (CAD) was developed and validated for the quantitative determination (assay) of 4-Methanesulfonyl-piperidine (analyte). This starting material is used in the synthesis of the intermediate which is precursor to BI drug substance (two steps upstream from the final step). This compound has no ultraviolet (UV) chromophore and showed no retention on typical reversed phase column. Retention of such polar compounds usually requires ion-pairing reagents and highly aqueous mobile phases. In this case heptafluorobutyric acid was used as an ion pairing agent for increased retention.

P#23

Title: STRUCTURE ELUCIDATION OF AN PROCESS IMPURITY IN A P38 MAP KINASE INHIBITOR DRUG SUBSTANCE USING LC/MS AND NMR

Authors: F. Qiu; S. Campbell; A. Granger; Z. Tan; J. Song; D. L. Norwood
Institution: Boehringer Ingelheim Pharmaceuticals, Inc.

Pharmaceutical impurity identification is not only a regulatory requirement, but also a critical problem-solving tool during chemical process development and pharmaceutical product development. Elucidation of the chemical structure of an unknown by-product or a degradation product is the first step toward understanding of the formation chemistry of the impurity and subsequent process and formulation optimization. The presentation gives a case study where an unknown process impurity was observed in a P38 MAP Kinase Inhibitor drug substance. The level of the impurity is at about 0.2% at release, which is above the ICH identification threshold for impurities in new drug substances. After a multidisciplinary analytical investigation, the chemical structure of the impurities was fully characterized, as shown below. This presentation will describe the details of the structure elucidation process using LC/MS, isolation and NMR and discuss the formation mechanism of the impurity.



P#24

Title: Retained Diluent Chromatography: A Revolutionary New Variation of HPLC

Authors: E. Loeser; S. Babiak
Institution: Novartis Pharmaceuticals Corporation

We have found that water-immiscible solvents can be used as satisfactory diluents in RP-LC, provided that the solvent used as diluent is retained more strongly by the column than the analytes in the sample. The elution of the analyte before the diluting solvent is the opposite of traditional HPLC, where the analyte elutes after the diluting solvent. We refer to this variation as “retained-diluent” HPLC. The theory and applications are discussed.

P#25

Title: Gravimetric Measurement of Excess Adsorption in a Binary Solvent System

Authors: E. Loeser; S. Babiak; Z. Liu
Institution: Novartis Pharmaceuticals Corporation

For binary solvent systems in contact with a solid adsorbent, excess adsorption of one solvent component over the other has been previously measured by a variety of methods. In this study, the feasability of using gravimetry to measure excess adsorption is explored, using a C18 derivatized silica gel HPLC column. The solvent system water + acetonitrile was used, because it is known to exhibit significant excess acetonitrile adsorption. Excess adsorption values obtained using the described gravimetric method compared favorably with literature values obtained using a different method.

P#26

Title: Utilization of UHPLC in Pfizer Groton

Authors: C. G. Cheng
Institution: Pfizer Inc

This poster presentation describes how Pfizer Analytical R&D utilizes UHPLC to improve productivity in the analytical laboratory. Pfizer AR&D not only has taken advantage of the speed and resolution advantage of the new instrumentation, but has also designed method development strategies around the instrument to further streamline the work flow in various groups. Specific examples as well as some practical concerns with the instrument will also be discussed in this presentation.

P#27

Title: Utilizing Fused-Core Technology for LC-MS Applications

Authors: Hillel K. Brandes, Craig R. Aurand, David S. Bell, Richard A. Henry, Wayne Way, Russel Gant, and Paul Ross
Institution: Supelco, Div. of Sigma-Aldrich, Bellefonte, PA 1682301

In this study an examination of fast chromatographic separations using the fused core technology with respect the to LC-MS analyte detections is made. A comparison of mass spectral sampling rates and scan speeds with ion trap and time of flight mass spectrometers are determined with respect to chromatographic peak resolution and sensitivity. This study illustrates the need for special consideration with respect to chromatographic run times and data acquisition rates and scan rates when developing fast LC-MS methods.

- Fast LC-MS with Ascentis Express mandates attention to instrument configuration parameters that effect data acquisition both for UV and MS detection.
- Scan range, trap fill times, sampling rates and scanning speeds all require considerations for to more accurately reflect the actual chromatographic resolution.
- Goal objectives such as sensitivity, speed or spectral quality need to be considered during LC-MS method development.

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