This October 19th, **Phenomenex** will be hosting the **North American PhenoPrep Seminar** in Princeton, NJ to discuss innovations and challenges in preparative and process scale chromatography. It will include a powerful dialog with industry leading experts, learning from them how they are tackling challenges, overcoming obstacles, and using innovative technologies.

This amazing gathering will host several speakers all discussing a variety of topics ranging from chiral chromatography, SFC, small scale purification, peptides and oligonucleotides, SFC, SMB, scale-up, software and instrumentation.

Want to learn more and register? Check it out now at:

www.phenomenex.com/PrepNa17
Interested in who will be speaking and what you can learn? Here is a quick rundown of the speakers and what they will be discussing at PhenoPrep.

Professor Bezhan Chankvetadze, Univ. of Tbilisi

“Continuous Chromatography Development Considerations from Discovery to Commercial Scale”

Polysaccharide-based chiral stationary phases offer number of advantages for liquid-phase separation of enantiomers such as universality, multimodal applicability, suitability for enantioseparations as with pressure-driven, as well as voltage-driven methods, high selectivity of separation for a certain group of chiral compounds, availability of large family of chiral selectors from several suppliers and applicability not only for analytical, but also for a

preparative and product-scale separation of enantiomers.

The first part of this presentation will discuss some unusual effects of the mobile phase composition (polar modifiers and additives) and temperature on separation of enantiomers with Lux series of chiral stationary phases. The second part will focus on the new members of Lux series of packing materials with the emphasis on additional opportunities these materials may offer not only for analytical, but also for preparative and product scale separation of enantiomers. To close the presentation, he will speak on the materials and technologies of the future, such as core-shell silica-based chiral stationary phases, lab-on a chip separation of enantiomers, and ultrafast separation of enantiomers.

Christina Kraml, Lotus Separations

“HPLC/SFC Purification and Characterization of Bacterial Signaling Molecules”

The isolation of small components from complex mixtures is a critical step in the identification of unknown target molecules and it commonly involves some type of chromatography. The overall strategy typically requires a large amount of ‘feed’ containing the product of interest (POI) and a definitive detection method such as an activity assay. This supports the development of purification methods and ultimately leads to the isolation of material for structure elucidation. As a final step, the POI is synthesized and its activity is evaluated.

Over the years, in support of Prof. Bonnie Bassler’s research, Kraml’s lab has been involved in the hunt for targets specifically produced by bacteria. Many bacterial species use a cell-cell communication known as quorum sensing to monitor cell population density and to control

cell behaviors. Quorum sensing relies on the production, release and detection of extracellular signals called autoinducers. Two of these signals, AI-2 and CAI-1, have already been identified and characterized by the Bassler and Semmelhack groups at Princeton University. Kraml will describe their contributions to the isolation, purification and characterization of CAI-1 and most recently of a new AI-2 mimic produced by yeast. The latest mimic is the first example of prokaryotic and eukaryotic cross-kingdom communication.

Christine Aurigemma, Pfizer, Inc.

“SFC/HPLC Screening for Small Molecule Purifications; Software Applications to Improve Workflow Efficiency”

A seamless and efficient purification process was developed by combining automation and informatics into a streamlined and simplified workflow. The full implementation of FastTrack screening, which utilizes both SFC and HPLC methods in a single system, has enabled the rapid identification of suitable separation conditions for achiral and chiral small molecule applications. Automated data processing further accelerates the transfer of samples from the method development stage to purification. Additionally, the embedded sample submission process as well as the capture of analytical data within the electronic notebook has resulted in efficiency improvements from sample submission to the documentation of data. This presentation will discuss this enhanced purification workflow.

Dr. Manny Ventura, ChemPartner

“Utilizing SFC for Separation of Peptides of Pharmaceutical Interest Generated from Automated Synthesis”

The design of bioactive peptides is of great importance to today’s pharmaceutical industry. Such custom peptides can be efficiently produced utilizing automation combined with certain suitable off-line synthesis steps. As the process becomes more efficient and productive there is a growing need to rapidly isolate the designed peptide of interest from the associated swath of impurities. Frequently such separations become too challenging with traditional reversed phase HPLC.

The present study will demonstrate how SFC can be utilized to provide the alternate selectivity required to isolate the peptide of interest from a complex mixture or provide the complementary selectivity to HPLC necessary to reach the final required purity when combined with an HPLC separation step. Using SFC for the purification of peptides also provides a timesaving advantage to the synthesis process through reduced method development time and shorter cycle time along with the reduced level of water in the mobile phase. Applications of preparative SFC demonstrating these advantages for certain mixtures will be described.

In addition, analytical SFC/MS can reveal impurities unresolved and not detected through standard LC/MS methods used to analyze peptide synthesis products, and examples demonstrating this will be shown. Separations exploiting the divergent selectivity of SFC for various peptide sequences from automated synthesis will be presented.

Dr. Matteo Villain, VP of R&D, Bachem Americas

“Seeking Alpha in Peptide Purification : API and Impurities Chemical Properties Should Drive the Peptidic API Purification Strategy Development”

Peptides are becoming an increasingly attractive target for API development in multiple therapeutic areas.

Bachem is one of the world leaders in industrial production of peptides using chemical synthesis. Production of peptides as APIs at industrial scale presents some specific challenges at the purification stage. The use of RP-HPLC is considered the industrial standard for peptide purification. Many factors in RP-HPLC influence alpha (selectivity factor). The chemical structure of the target API and of the impurities is one of these factors. The presentation will provide a brief overview of peptide chemicophysical properties and how they influence the purification process development. Some emphasis will be given to the importance of early analytical method development and the application of UPLC as the primary tool for monitoring the purification process. The importance of UPLC-MS as tool in the purification development will be also part of the presentation.

Dr. Ben Hritzko

“Peptide Purification: Bridging the Gap Between Discovery and Development”

Production of high purity synthetic peptides can be a challenging effort due to the complexity of the mixtures and a heavy reliance on chromatographic resources. In Discovery, the requirement to quickly evaluate candidates with limited resources favors the implementation

of platform purification technologies. In Development, the requirement to produce larger quantities under cGMPs carries additional challenges. By coupling the use of internal and external resources, the process can be streamlined while providing learning opportunities along the way. In this presentation, we report on strategies that were employed to efficiently move critical targets through Discovery and Developmentphases.

Dr. Zina Sergueeva, Cepheid

“Preparative Purification of Synthetic Oligonucleotides for Diagnostic Use”

Oligonucleotides are polymeric sequences of nucleotides (RNA, DNA, and their analogs) that are extensively utilized in life science research, in PCR-based diagnostic test kits, and more recently as direct therapeutic agents against a wide range of disease conditions.

While purification of therapeutic grade Oligonucleotides now receives all the attention, preparation of Oligonucleotide analogs for diagnostic applications presents a very distinct range of issues. Introduction of fluorescent dyes and quenchers with complex chemical structures, various nucleoside modifications and partial self-complementarity of oligonucleotide analogs requires intensive HPLC method development.

Current presentation is focused on several recent key improvements of purification process developed for synthetic oligonucleotides with Cepheid proprietary moieties. Firstly, we have implemented a heating system for preparative scale columns that allows us to resolve oligonucleotide secondary structures that frequently impede separation of synthetic impurities. For efficient separation of target oligonucleotides with the simultaneous removal of protecting groups we implemented rarely used technique that works well with a certain

type of Reverse-Phase columns. Finally, some details of solid phase selection for purification of oligonucleotides with Cepheid proprietary highly hydrophobic moieties will be discussed in the presentation.

Dr. Mirlinda Biba, Merck

“Analysis and Preparative Purification of Oligonucleotides”

Synthetic oligonucleotides (about 20-25-mers) have become increasingly important as part of antisense and short interfering ribonucleic acid (siRNA) drug development efforts for the treatment of different diseases including cancer and viral infections. The natural and chemically modified oligonucleotides can be readily prepared by automated chemical synthesis, but with limited purity. The development of analytical methods for the sensitive and quantitative analysis and separation of oligonucleotides is an essential part for the advancement of this research area. Additionally, these oligonucleotides must be purified prior to use to avoid any off-target silencing effects. The purification methods typically include ion-pair reversed-phase liquid chromatography (IP-RPLC) or strong-anion exchange liquid chromatography (SAX-LC). In this presentation, numerous examples for purification of different natural and modified 21-mer RNA oligonucleotides from milligram to gram scale by IP-RPLC will be illustrated.

Dr. Henri Colin, Novasep

“Fundamental Aspects of Supercritical Fluid Chromatography and Specific Considerations for Preparative Applications”

If (modern) Preparative Liquid Chromatography (PLC) have been accepted as an inevitable production tool in the pharmaceutical industry for many years, the replacement of the liquid phase by supercritical CO2 is still not so well accepted and/or recognized. Several factors can explain this: the price of the (sophisticated) technology, the fear of supercritical CO2 (it is definitely not as simple to use as a “normal” liquid), and other more or less justified “a-priory”.

This is unfortunate because supercritical CO2 offers very significant advantages compared to the standard solvents used in PLC, two of the most important ones probably being (1) its low viscosity and (2) its low price. This is of particular important when one keeps in mind that (i) the productivity of a preparative chromatography process is almost inversely proportional to the viscosity of the mobile phase (everything else being constant); and (ii) typically 70 % of the process cost comes from the mobile phase (even when it is recycled).

The lecture will address various theoretical aspects of the use of supercritical CO2 (including its limitations), the specificities of the technology to use efficiently this fluid, and some practical examples of applications.

Dr. Olivier Dapremont, AMPAC

“Continuous Chromatography Development Considerations from Discovery to Commercial Scale”

Continuous chromatography using simulated moving bed technology (SMB) has been used for the purification of chiral APIs in the pharmaceutical industry for over 25 years. The process is very competitive compared to other techniques due to its continuous nature and the inherent solvent efficiency of the process (99.98% solvent recycling at commercial scale) making it a Green Process. The technology has reached maturity and has been inspected and approved by the FDA for the production of several APIs. Currently, SMB is also being evaluated for non-chiral separations and unusual or difficult purification processes. Regardless of the separation, the development process remains similar and lessons learned on one compound can be applied to the next candidate. Through examples, we will walk through the process of developing a separation using SMB to produce APIs from lab scale (kgs) to implementation at commercial scale (multi-tons).

Dr. J Preston, Phenomenex

“Pick the Right Tool from the Preparative Chromatography Toolbox”

A simplified definition of chromatography is the separation of a mixture by passing it through (or over) a medium in which the components move at different rates. This concept is over 100 years old and modern drug development would not exist without it. Preparative HPLC is similar to analytical HPLC in many aspects but there are significant differences. It is simple to define the goal of prep chromatography, “to isolate material from a mixture.” However, it can be very difficult to describe the application of prep chromatography. Many tools have been developed to cover the extensive landscape of prep chromatography. The desired material can vary from small organic compounds to very large biomolecules and the required scale can range from mg to metric tons. The basic concepts are consistent, where each project is a balancing act for the triangle of purity, yield and throughput.

The work presented here will use several pharmaceutical relevant examples to demonstrate strategies from the “Chromatography Toolbox” for the development of preparative methodologies. These examples will include achiral purification methodology for peptides and small molecules along with chiral methodology for small molecules. The examples will highlight the concepts of column screening, eluent selection, phase appropriate method optimization, multi-step purifications, material isolation techniques and yield estimation during development.

DJ Tognarelli, JASCO

“Improving Chiral and Achiral Throughput with Parallel and Open-Bed Preparative SFC”

Chiral and achiral SFC analysis requires solvent and column screening in order to determine the best solvent-column combination and provide the desired resolution for the peaks of

interest. With the increasing number of chiral and achiral columns and the increase in the number of samples to analyze, higher throughput is required. Scaling up this higher throughput to preparative requires flexibility for repeat and stacked injections, but also for multiple samples for open-bed mass spectrometer triggered collection.

In this discussion I will show the parallel SFC for higher throughput in solvent and column screening for chiral and achiral analysis. The parallel SFC offers the ability to run several column and solvent combinations. As many as 10 solvents and anywhere from 5-25 columns can be configured with the system, but also the ability to optimize the separation on a single column after the parallel screen. The use of the preparative SFC with open-bed fraction collection with mass spectrometer triggering is explored to increase the number of fractions for multiple peaks and multiple samples, still yielding high purity and high recoveries.

We hope to see everyone there for the 2017 North American PhenoPrep Seminar on October 19th in Princeton, NJ!

If you have any questions or wish to register, visit the site now!

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Summary

Article Name
Join Us for the North American PhenoPrep Symposium
Description
Join us October 19th for the North American PhenoPrep Symposium for talks on innovations and challenges in preparative and process scale chromatography