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Scientific Tracks & Abstracts

(Day 1)

Yukihiro Shoyama, J Chromatogr Sep Tech 2016, 7:5(Suppl)
http://dx.doi.org/10.4172/2157-7064.C1.016

Fingerprinting of natural product by eastern blotting using monoclonal antibodies

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We prepared many kinds of monoclonal antibodies (MAbs) against natural products and developed a new staining method using MAb named as Eastern blotting. Glycosides like ginsenoside were developed by TLC, and the TLC plate was covered by PVDF or PES membrane and blotted. The membrane was treated with NaIO4, and then with carrier protein resulting in glycoside-carrier protein conjugates on membrane. In the case of non-glycoside natural product like aristolochic acids, the conjugate with carrier protein was synthesized by appropriate pathway on the membrane. Peroxidase labeled secondary MAb and then substrate were added, successively. Several ginsengs were analyzed to find out unknown ginsenosides in American and Japanese ginseng by this fingerprinting. Also we separated ginsenosides using affinity column combined MAb from the crude extract. The other bioactive glycosides like saikosaponins and glycyrrhizin can be stained and applied for fingerprinting analysis. Aristolochic acids having kidney toxicity in *Alistolochia* spp. were separated by TLC and then blotted onto a PES membrane by employing a modified carbodiimide method. The resulting membrane-bound aristolochic acids-protein conjugates can be stained by Eastern blotting and fingerprinting. The staining of aristolochic acid in mouse kidney tissues was succeeded. Moreover, we detected sequensed and determined a target protein against aristolochic acid in mouse kidney cell lines using anti-aristolochic acid MAb. These related results will be also discussed.

Biography

Yukihiro Shoyama worked in MGH as a Post-doctor in 1975. During 1978 to 1991, he worked as an Associate Professor and as a Full Professor during 1991 to 2007 in Kyushu University. During these periods he managed as the director of Pharmacognosy department, the director of herbal garden and the dean ship (2004-2006). He moved to Faculty of Pharmaceutical Sciences, Nagasaki International University as a Full Professor from 2007. He had efforts for the President of Japanese Society of Pharmacognosy (2007-2008) and Vice Chairperson of Specialty Committee of TCM Pharmaceutical Chemistry of World Federation of Chinese Medicine Societies (2012). His research interests are marihuana studies, monoclonal antibodies against natural product, biotechnology of medicinal plants and bioactive natural products.

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Notes:

Kamal Matar, J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Topiramate quantitation in human plasma by liquid chromatography-tandem mass spectrometry: Application to therapeutic drug monitoring

Kamal Matar
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Background: Topiramate (TPM) is a new antiepileptic drug (AED) used worldwide in patients with various types of epilepsies and also for prophylaxis of migraine. A rapid, selective, reliable, precise, accurate, and reproducible liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for quantification of TPM in human plasma using topiramate-d12 as an Internal Standard (IS) has been developed and validated to be used routinely for TDM of TPM.

Methods: The drug and IS were extracted by ether and analyzed on Symmetry® C18 column. Quantitation was achieved using ESI- interface employing MRM mode.

Results: The method was validated over the concentration range of 0.5–30 µg/ml (r>0.99). Intra- and inter-run precisions of TPM assay at three concentrations ranged from 0.7 to 7.8% with accuracy (bias) varied from −10.0 to 2.1% indicating good precision and accuracy. Analytical recoveries of TPM and IS from spiked human plasma were in the range of 84.1 to 90.0% and 90.0 to 111.0%, respectively. Stability of TPM in human plasma samples at different conditions showed that the drug was stable under the studied conditions. Matrix effect study showed a lack of matrix effect on mass ions of TPM and IS.

Conclusions: The described method compared well when assessed by LGC TDM theme program (r>0.99). The suitability of the developed method for TDM was demonstrated by measuring TPM in human plasma samples of epileptic patients treated with TPM. The proposed method is appropriate for routine TDM of TPM.

Biography

Dr. Kamal Matar is an Associate Professor and Chairman of the Department of Pharmacology & Therapeutics. He is also a director of Therapeutic Drug Monitoring & Clinical Toxicology (TDM&CT) Unit, Faculty of Medicine, Kuwait University. He has published over 35 peer-reviewed articles and over 20 abstracts in international conferences. He is serving as a reviewer for some international journals such as: Journal of Chromatography B, Journal of Pharmaceutical & Biomedical Analysis, Chemotherapy, Drugs, Analytical Chemistry Insights and Journal of Antimicrobial Chemotherapy. He is a member of International Association of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), American Association of Clinical Toxicology (AACT), and European Compliance Academy (ECA). He organized many International Conferences in Kuwait in the area of Population Pharmacokinetics and Clinical Toxicology.

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Notes:

Ahmed Mohamed Ali Hemdan et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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New applications of reversed phase chromatography for detection and quantitation of certain adulterants in illegal herbal medicines

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A reversed phase HPLC method with UV detection has been developed and subsequently validated for the determination of some adulterants in illegal herbal medicines. Five herbal medicines for weight loss and other one for erectile dysfunction were tested for the presence of possible adulterants. The weight loss products were found adulterated with the already withdrawn drug Sibutramine and with Phenolphthalein that is proved to cause tumors. On the other hand, Sildenafil, a contraindicated drug for patients with heart diseases, was found in the herbal products for erectile dysfunctions. The methods use Inertsil C18 column (250x4.6 mm, 5 µ) and a flow rate of 1 mL/min. The mobile phase for quantitation of Sibutramine and Phenolphthalein was consisting of acetonitrile-potassium hydrogen phosphate buffer pH=3 adjusted by o-phosphoric acid (40/60 v/v) and detection at 223 nm. While the mobile phase for Sildenafil was acetonitrile-potassium hydrogen phosphate buffer pH=3.2 adjusted by o-phosphoric acid (50/50 v/v) and detection at 230 nm. The confirmation of the presence of the adulterants was done using LC-PDA and MASS spectrometry. For this, a complete investigation of any herbal medicine should be done through orthogonal analysis methods before use even if it is labeled with (100% herbal content).

Biography

Ahmed Mohamed Ali Hemdan has completed his PhD from Ain Shams University. He has published more 10 papers in reputed journals.

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Notes:

Evelien Wynendaele et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Implementation of UHPLC-single quad MS platform: The next level transforming pharmaceutical quality control laboratories

Evelien Wynendaele, Gevaert Bert, Veryser Lieselotte, Xu Xiaolong, Verbeke Frederick and De Spiegeleer Bart
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In pharmaceutical analysis, the use of mass spectrometry (MS) coupled to liquid chromatography in quality control has become very significant. This is observed in i. a. the European Pharmacopoeia, where this technique is already incorporated in different general texts as well as specific monographs. Here, often (GMP) single quad MS platforms can be used, characterized by acceptable low costs, easy-to-use smooth lab-integration, increased efficiency and providing additional detection possibilities over a traditional UV/VIS detector. Single quad mass spectrometers also work well as analytical tools for the detection of low-abundant genotoxic impurities and their basic confirmation. Moreover, also in drug development, this detector can be useful: low UV-detectable impurities, obtained during synthesis, formulation or after degradation, can be analyzed, even in complex matrices. In developmental quality control of complex mixtures (e.g. plant extracts), the UPLC-single quad MS platform was found to be of great use in transdermal research as well.

Biography

Evelien Wynendaele has completed her PhD in Pharmaceutical Sciences in 2014 at Ghent University. She is currently working as a Post-doctoral fellow in the DruQuaR Laboratory at the same university, under the supervision of Prof. Bart De Spiegeleer. She has published more than 35 papers in reputed journals.

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Rafea M Naffa et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Analysis of natural crosslinks by liquid chromatography mass spectrometry using a silica hydride column

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The natural crosslinks between tropocollagen molecules in skin contribute to its physical properties of strength and flexibility. Despite advances in analytical techniques such as mass spectrometry, the methods used for their analysis and quantitation date back to the 70s through to the 90s, are challenging and time consuming. As a result, it is difficult to obtain standards to verify analyses of these compounds. We have developed methods to isolate highly pure, natural crosslinks from skin, and have verified their structures using mass spectrometry and NMR. Fragmentation studies of these crosslinks will enable the development of a method for label-free quantitation in skin hydrolysates. We have also developed a novel chromatographic analytical method for the simultaneous determination of natural crosslinks. Seven crosslinks were separated on Cogent Diamond Hydride HPLC column using isocratic and gradient conditions then detected by mass spectrometry without derivatization. Total run time of less than 10 minutes was achieved under isocratic conditions using water and acetonitrile. To the best of our knowledge, this is the first method in which histidinolysinonorleucine (HHL) and histidinohydroxymerodesmosine (HHMD) were separated and identified by the mass spectrometry. This technique was applied on skin, elastin and cartilage in which strong evidence suggested the presence of undocumented crosslinks. The developed method will be widely used for quantitative and qualitative analysis of natural crosslinks in biological samples as well as characterization of new crosslinks.

Biography

Rafea M Naffa is pursuing his PhD from Massey University, New Zealand. He has worked as Lecturer at University of Sharjah from a period of 2007-2014. He completed his Master's in Chemistry from Hashemite University. His research interest includes Chemistry, Bio-Analytical Chemistry and Leather Chemistry.

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Notes:

Britta Eggenreich et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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A novel and fast approach to monitor cell disruption efficiency

Britta Eggenreich, Vignesh Rajamanickam, Christoph Herwig and Oliver Spadiut
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The bacterium *Escherichia coli*, is a well-studied recombinant host organism with a plethora of applications in biotechnology. High valuable biopharmaceuticals, such as recombinant enzymes, antibody fragments and growth factors, are currently being produced in *E. coli.* These molecules are usually produced intra-cellularly which is why cell disruption is required as the first step in the downstream process. For that purpose, high pressure homogenization is the system of choice since it is scalable and can be run in continuous mode. However, it is crucial to determine cell disruption efficiency to: Avoid product loss in intact cells, but also to avoid unnecessary long disruption cycles and thus harm the product. Usually, cell disruption efficiency is evaluated either by determination of colony forming units (CFUs) or photometric measurements of nucleic acids and protein content in the lysate. However, these methods are both characterized by disadvantages, as CFUs can only be counted on the next day, resulting in great time delay, and photometric measurements are affected by matrix effects. In this study, we implemented a novel online tool based on UV chromatogram fingerprints and chemometric techniques to monitor cell disruption efficiency. We used: 1) Measurement of the total protein content in the supernatant, 2) determination of CFUs and 3) flow cytometry as reference analytics to validate this novel tool. Finally, we performed a design of experiments study, where we changed the factors concentration of biomass per ml buffer, number of homogenization cycles and pressure during homogenization to analyze and optimize the unit operation high pressure homogenization for a recombinant E. coli strain producing a highly valuable antibody fragment. Summarizing, we could nicely demonstrate the power of the novel online tool, which will certainly facilitate the evaluation of this crucial unit operation in the future.

Biography

Britta Eggenreich has finished her studies of Pharmaceutical Science at the University of Vienna in the year 2011. She has worked for two years as a Pharmacist in Vienna. In 2014, she started her Doctoral thesis in the group of Biochemical Engineering at Vienna University of Technology. Currently, her main focus is the downstream development for inclusion bodies of a novel antibody fragment produced in *E. coli.*

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Quantification of drug metabolites in the absence of authentic standards

Filip Cuyckens[1], Balázs Klencsár[2], Valerie Koppen[1], Ronald de Vries[1], Cis Van Looveren[1], Bjorn Meermann[2], Lieve Balcaen[2] and **Frank Vanhaecke[2]**

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Quantification of metabolites in the absence of an authentic standard is a challenging task. Metabolites are usually present in relatively low concentrations residing in a large background of endogenous compounds and their MS response factor can significantly differ from the parent molecule. The use of a radiotracer overcomes these challenges and, therefore, remains the method of choice for quantification of metabolites in complex matrices, but is not always available or cannot always be applied. Typically samples from first-in-human studies are not radioactive but still extremely valuable giving a first insight in human metabolism. Therefore, estimation of metabolite abundance in these samples is important and also recommended by regulatory guidelines (ICH M3).

An overview will be given of different established and novel approaches for the quantification of metabolites in *in vitro* and *in vivo* matrices in the absence of authentic standards. The following techniques will be discussed: radioactive detection[1], Accelerator Mass Spectrometry (AMS), UV detection, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)[2-4] and Electrospray Ionization-Mass Spectrometry (ESI-MS) using matrix mixing[5] or a $^{12}C/^{14}C$ isotope ratio approach[6].

Depending on the circumstances (sample volume, sample matrix, compound structure, question to be answered, availability of a radiolabel, etc.) the right tool or combination of tools need to be selected since none of these techniques should be seen as the standard technique that suits all measurements.

Biography

Filip Cuyckens is a Scientific Director & Fellow at Janssen R&D in Beerse, Belgium. He is responsible for Analytical Sciences in the Pharmacokinetics, Dynamics & Metabolism (PDM) department. Analytical Sciences PDM consists of Biotransformations, focusing on metabolite profiling and identification of discovery to late development compounds, and Discovery & Exploratory Bioanalysis, focusing on quantification of drug candidates, metabolites and biomarkers in biological matrices. Filip earned a pharmacist degree in 1998, a degree in industrial pharmacy in 2002 and a Ph.D. in pharmaceutical sciences in 2003.He has (co)authored more than 50 publications, is a member of the associate editorial board of Rapid Communications in Mass Spectrometry and board member of the Belgian Society for Mass Spectrometry.

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Siu-Po IP, J Chromatogr Sep Tech 2016, 7:5(Suppl)
http://dx.doi.org/10.4172/2157-7064.C1.016

The development of standards for quality control of herbal medicines

Siu-Po IP
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Herbal medicines have been used for thousands of years and they are recognized as safe and effective remedies for the prevention and treatment of diseases. However, the herbal materials are usually obtained from natural sources and they are often criticized for their lack of quality control. The quality of herbal drugs can be affected by many factors including source, geographic location, growing conditions, harvesting season, and processing procedures. Therefore, it is necessary to develop regulatory standards to control the quality of herbal medicines. The Hong Kong Chinese MateriaMedica Standards (HKCMMS) project was launched in 2002 by Department of Health, HKSAR Government to develop standards for commonly used herbal drugs to ensure the safe use and the quality of the drugs. Now, it has become widely recognized reference standards for Chinese medicine traders, laboratories in the field of Chinese medicine testing and certification, and some overseas organizations. The HKCMMS project is under the guidance of the International Advisory Board which consists of international renowned experts. The research works for the development of chromatographic methods are conducted by research teams from seven local and overseas research institutes and the National Institutes for Food and Drug Control, China. Our research team had participated in the HKCMMS project since its beginning and contributed significantly to the successful implementation of the project. Up to now, 236 monographs were published in 7 volumes of HKCMMS. Among of them, the research works of 47 monographs were totally contributed by our research team.

Biography

Siu-Po IP received his PhD degree in the Department of Biochemistry, The Hong Kong University of Science and Technology. Currently, he is working as a Research Fellow in the School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong. He is also appointed as a member of Examination Committee, The Chinese Medicine Council of Hong Kong and a Technical Assessor of Hong Kong Accreditation Service. He has extensive research experience on herbal medicines. He has published about 100 papers in reputed journals.

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Study on the generation mechanism and change regularity of characteristic "raisin" flavor compounds in raisins

Dong Wang, Jun Wang and Ying Shi
China Agricultural University, China

Raisins are a welcome dry fruit all around the world. They are either consumed directly or are used in cooking especially in desserts. The aroma of raisins affects consumers' preferences. The aroma of raisin is determined by grape variety, drying method and storage environment. Over 100 volatile compounds were identified in different raisins by using gas chromatography-mass spectrometry (GC-MS) coupled with headspace solid-phase micro-extractions (SPME), the majority of raisin volatiles are derived from three sources: fresh grapes, the oxidative degradation of unsaturated fatty acids and the Millard reaction process. Most of the volatiles existed in fresh grapes, while the furans (5-methyl furfural, 2-acetyl furan 2-pentyl furan) and pyrazines (2-ethyl-6-methyl pyrazine, 2, 6-diethyl pyrazine and 5-ethyl-2,3-dimethyl pyrazin) generated by Maillard reaction during grape drying, they contribute to characteristic "raisin" flavor, and determine the aroma characteristics of raisins. This program will study the evolutionary pattern of these volatiles responsible for the characteristic "raisin" flavor and their precusors (amino acids and monosaccharides) during grape drying by integrating GC-MS, HP-LC, model reaction and metabonomics technology. Then illustrate the generation source and reaction mechanism of the characteristic volatiles and dissect the influence of drying methods, storage packaging form and environment conditions on these volatiles. The results will provide strong academic support and practical technology for manipulation of the flavor of raisins.

Biography

Dong Wang is a PhD candidate in China Agricultural University, majoring in Food Biotechnology. She studied in College of Food Science and Nutritional Engineering of China Agricultural University from the year of 2007 until now. As the leader of the research group, she has studied raisins flavor, amino acid, sensory evaluation, browning mechanism and quality characteristic of raisins for 6 years and published 3 papers in reputed journals.

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Katsuhiro Maeda, J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Elution order switchable chiral stationary phases for HPLC based on switching and memory of helicity in polyacetylenes in the solid state

Katsuhiro Maeda
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Separation of enantiomers by high-performance liquid chromatography (HPLC) is an effective method both for analyzing enantiomer composition of chiral compounds and obtaining pure enantiomers. Although a large number of chiral stationary phases (CSPs) for HPLC have been developed, it is still a challenging issue to switch the elution order of enantiomers under identical chromatographic conditions. Recently, we have found that a polyacetylene derivative bearing 2,2'-biphenol-derived pendants can form a preferable helical conformation in response to the chirality of non-racemic guest compounds, such as 1-phenylethanol (PEA), in the solid state as well as in solution, and the induced preferred-handed macromolecular helicity can be maintained, that is memorized, even after complete removal of the chiral guests. By taking advantage of this unique feature, we have succeeded in developing an unprecedented switchable CSP for HPLC, in which the elution order of the enantiomers can be switched, which will be based on reversible switching and subsequent memory of the macromolecular helicity by the treatment with (R)- and (S)-PEA in the solid state. In order to improve the chiral recognition ability, we synthesized analogous polyacetylene derivatives with ester or carbamate groups as the effective interaction sites and investigated a relationship between the structures of the pendants and the recognition abilities of the polymers. Repetitive switching of the elution order of enantiomers based on the switching of the macromolecular helicity was achieved by immobilizing these polymers onto silica support.

Biography

Katsuhiro Maeda has received his BS (1993), MS (1995) and PhD (1998) degrees from Nagoya University. In 1998, he joined the Graduate School of Molecular Design and Engineering, Nagoya University, as an Assistant Professor and was promoted to an Associate Professor in 2002. He moved to Kanazawa University in 2008 and was appointed as a Full Professor in 2015. He has published more than 80 original papers in various reputed journals.

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Michael Soll et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Biomass upgrading and catalyst characterization using a tandem reactor - GC/MS system

Michael Soll, Robert Freeman and Chu Watanabe
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The promise of converting various biomass feed stocks directly to biofuels or value-added specialty chemicals using catalytic pyrolysis has precipitated a demand for a fast, reliable method to characterize newly developed catalysts. Catalytic pyrolysis is a three-step process: (1) the feedstock is first pyrolyzed (which is often referred to as "fast pyrolysis"), (2) the pyrolyzates flow through a catalyst bed and (3) the 'products' are identified and quantitated. The tandem micro-reactor GC/MS system integrates these three processes into a single bench-top instrument. The tandem catalytic reactor is designed for the rapid evaluation and characterization of catalysts in various atmospheres, at different temperatures and pressures. Three modes of operation allow different experiments to be conducted on the same equipment. The GC/MS can operate in an on-line MS mode for continuous analysis of gases from the catalyst bed. Alternatively, the GC/MS can operate in several integrated high resolution GC/MS modes for step-wise experiments on gases exiting the catalyst bed. There is also a flash vaporization mode used for the pyrolysis of a solid sample. The system consists of an upper micro-furnace and lower micro-reactor each with independent temperature and reaction gas controls. The micro-furnace can accept solid or viscous liquid samples in a batch sampling mode with catalysis occurring under different conditions in the micro-reactor. The micro-reactor is designed to allow a quick change of the catalyst bed. Batch or continuous experiments can be performed with this system to evaluate both catalyst performance and to characterize catalysis products. Each capability outlined above will be illustrated using ethanol, wood flower and a variety of catalysts. By adding a newly developed Medium Pressure Flow Controller, the influence of reaction pressure against the rate of glycerine conversion using a palladium catalyst is also demonstrated.

Biography

Michael Soll has completed his PhD in Biology in 1993 at RUB, Germany. Since more than 20 years, he is working in business development, marketing and sales of GC- and LC-MS based laboratory equipment. Since 2014, he is representing Frontier Laboratories Japan in Europe as Business Development Manager.

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A novel toolbox with chromatogram fingerprints for lysis monitoring in *E.coli* bioprocesses

Vignesh Rajamanickam, David Wurm, Christoph Herwig and Oliver Spadiut
Vienna University of Technology, Austria

The bacterium Escherichia coli is a well-studied recombinant host organism with a plethora of applications in biotechnology. High valuable biopharmaceuticals, such as antibody fragments and growth factors, are currently being produced in E. coli. However, the high metabolic burden during recombinant protein production can lead to cell death, consequent lysis and undesired product loss. Thus, fast and precise analyzers to monitor *E. coli* bioprocesses and to retrieve key process information, such as the optimal time point of harvest, are needed. However, such reliable monitoring tools are still scarce to date. In this study, we cultivated a recombinant *E. coli* strain producing a recombinant single chain antibody fragment (scFv) in the cytoplasm. In bioreactor cultivations, we purposely triggered cell lysis by pH ramps. We developed a novel toolbox using UV chromatogram fingerprints and chemometric techniques to monitor these lysis events and used flow cytometry (FCM) as reference method to quantify viability offline. Furthermore, we tested the applicability of the novel toolbox for montiroing other *E. coli* bioprocesses. We are convinced that this toolbox will not only facilitate *E. coli* bioprocess monitoring, but will also allow enhanced process control in the future.

Biography

Vignesh Rajamanickam procured his Master of Science in Pharmaceutical Biotechnology from Hamburg University of Applied Sciences, Germany and, Bachelor of Technology in Biotechnology from Anna University, India. He started his PhD on March 2014 in Biochemical Engineering from Vienna University of Technology (VUT), Austria. Currently, he is working as a Project Assistant for developing a novel PAT tool for bioprocess monitoring and control in Christian Doppler laboratory for mechanistic and physiological methods for improved bioprocesses, VUT, Austria.

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Scientific Tracks & Abstracts

(Day 2)

Yun Fa et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Multi-dimensional ion chromatography–mass spectrometry for simultaneous determination of extracellular metabolites in Clostridium thermocellum

Yun Fa and Fangfang Li
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In bio-energy and bioprocess research, quantitative understanding of bacterial metabolism and determination of metabolic flux data are necessary, especially for the strain improvement, gene function analysis, optimizing the cell system and the production process, fermentation experiment design. It takes long time for so many metabolites because different methods are needed for different types of metabolites. Although traditional reversed-phase liquid chromatography tandem mass spectrometry (RPLC-MS) covered a wide range of small molecule metabolites, but some ions and polar compounds in the conventional reversed-phase columns do not retain or keep very weak, such as organic acids, sugar, sugar phosphate, amino acids and other. These compounds are just one of the main objects in the analysis of metabolomics. We have built a multidimensional ion chromatography - mass spectrometry combined system, and achieved simultaneous analysis for the polar group of amino acids, sugars, alcohols, organic acids, some important cations. Further, the accuracy and precision of the method were investigated. And the new method was successfully used to determine the target metabolites in extracellular culture media of Clostridium thermocellum for not more than 2h. Using a combined system target to metabolomic analysis, we provided the more exact experimental data for designing control process. The establishment of multidimensional ion chromatography tandem mass spectrometry platform can be used for analysis of water soluble metabolites not only in bio-energy research, but also in the field of other areas, such as food, environment and life sciences applications.

Biography

Yun Fa is an Associate Professor in key lab, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. She worked on ion-chromatography analysis for 7 years. She has published more than 15 papers in reputed journals.

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Notes:

Sena Yaman et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Dendron-based stationary phases for hydrophobic interaction chromatography

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Hydrophobic Interaction Chromatography (HIC) is a powerful technique used to purify proteins. It relies on the interaction between non-polar groups on the hydrophobic patches of the proteins and ligands on the HIC medium. This interaction varies according to the hydrophobicity of biomolecules, leading to the separation of proteins in a complex mixture. In this work, two novel stationary phases for HIC were synthesized by incorporating dendronitic structures, branched molecules. The branched nature of the dendrons allowed the attachment of high amount of hydrophobic ligands to the support material. Depending on the number of branches of the dendron used, ligand densities were calculated as 82.5±11 and 175.6±5.7 µmol ligand/ml resin for Sorbent 1 and Sorbent 2, respectively. UV-Vis absorption spectra of the modified sorbents exhibited a band at 287 nm corresponding to the aromatic ring present on the dendrons suggesting their incorporation onto the sorbents. FTIR analysis evidenced the aromatic and carbonyl groups suggesting the presecence of dendrons with hidrophobic ligands on the sorbents. Adsorption capacity of the sorbents was evaluated in static and dynamic mode using bovine serum albumin (BSA) under high concentrations of ammonium sulfate (AS). Increasing AS concentration from 1.5-2 M led to significant increases in adsorption capacity. Dynamic adsorption was influenced by flow velocity. This innovative design allowed to increase the ligand density and therefore the adsorption capacity of the sorbents. This technology may permit to reduce the amount of sorbent to be used in a bioprocess and thus use smaller columns resulting in faster chromatoraphic processes.

Biography

Sena Yaman has completed her MSc degree in Middle East Technical University and is currently working on her Doctoral studies in Izmir Institute of Technology, Department of Bioengineering.

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Separation of drug active ingredients by using subcritical water chromatography

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Drug active ingredients contain one or more chemical substances that cause physiological changes *in vivo* and substances that are responsible for the beneficial health effects experienced by patients. Drugs are usually grouped according to their physiological effects and antidepressants are one of the most frequently used of this group. In this study, fluoxetine and sertraline are used as selected model molecules obtained from antidepressant active agent, which have environmental as well as pharmacological and biological importance. For this purpose, naphthylamine attached poly (HEMA-MAH)(PHEMAH) (poly(hydroxyethyl methacrylate-N-methacryloyl-(L-histidine)-methylester) microspheres, which have different particle sizes will be synthesized and used as the column filling material (stationary phase) in high temperature liquid chromatography which is one of the green chromatography techniques and determination of the method development and optimization conditions for the synthesized column filler will be conducted by using chemometrics application of response surface methodology. Many analytical methods such as High Performance Liquid Chromatography, Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry, Gas Chromatography-Mass Spectrometry are used in literature for the chromatographic separation of the active ingredients of antidepressants. Among the used techniques, the High Performance Liquid Chromatography (HPLC) shows the most effective separation technique. While RPLC (Reversed Phase Liquid Chromatography) is a popular analytical technique used today, organic solvents are required in traditional RPLC and an enormous amount of organic solvent is consumed worldwide for just chromatographic separation. The organic solvents used in HPLC are very hazardous and dangerous for environmental and human health, as well as expensive in term of both purchasing and waste disposal costs. Industrial wastewater pollution is being one of the major and perhaps most importantly problem resulting from uncontrolled technology in developing countries. Therefore, separation, purification and determination methods are more important and "nature-friendly" products and dissemination of related researchers is of great importance for use as solid/solid phase of determination methods due to environmental concerns. High temperature liquid water chromatography technique used the minimum level of organic solvent in which worldwide attention has been obtained recently. At the same time, in order to be successful in the separation process in HTLC, new generation of stable and resistant filler column is synthesized and is also an important application which is successful at elevated temperature conditions. The systematic studies are rapid, accurate, is expected to reach accurate and reliable results as the requirement of a scientific research.

Biography

Berkant Kayan is an Associate Professor of Chemistry, Aksaray University, Aksaray, Turkey. He has joined Aksaray University in 2009 as Assistant Professor and then quickly promoted to the Associate Professor position in 2012. He has 18 publications and research grants and projects from Scientific and Technological Research Council of Turkey - TUBITAK, Aksaray University-ASU-BAP-2012-9, and Mersin University- MERSIN-BAP. He joined the Department of Chemistry at East Carolina University in 2005 as visiting doctoral student for four months which was supported by Mersin University. He visited Technological and Educational Institute of Crete, Chania, Crete, Greece (2013) and Université Paris-Est Marne-la-Vallée., Paris, France (2015) with European Erasmus Bilateral as Guest Academic Researcher.

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Implementation of ssDNA aptamers for imidazole-free purification of His_3-tagged recombinant proteins

Wojciech Strzałka
Jagiellonian University, Poland

The dynamic development of genetic engineering has opened new perspectives for the production of recombinant proteins which are currently offered by many biotech companies. In addition to the medical and industrial applications they are also extensively used in basic research studies. Recombinant proteins are often produced in heterologous expression systems, for example in *E. coli* cells. Before proteins find a final application, purification, a key stage of the production process, must be performed. Therefore affinity chromatography systems were developed for the fast and simple isolation of recombinant proteins. One of such systems is Immobilized Metal Ion Affinity Chromatography (IMAC), which is commonly used for the purification of His_6-tagged recombinant proteins. Although it is a powerful system it is not free of disadvantages. Recently an alternative solution, which is free of IMAC drawbacks, was developed. It is based on a unique ssDNA sequence, called the H_3T aptamer, which was selected for the purification of His_3-tagged recombinant proteins. The binding of the H_3T aptamer to His_3-tag is controlled by sodium ion concentration. Based on this feature H_3T aptamer resins can be successfully employed for the purification of His_3-tagged recombinant proteins from *E. coli* total protein extracts using imidazole-free buffers. The purity of His_3-tagged proteins is superior when purified with the help of the H_3T aptamer in comparison with IMAC resins.

Biography

Wojciech Strzałka has completed his PhD at the Jagiellonian University. He completed Post-doc at Salento University, Italy and Osaka University, Japan. Currently, he is a Group Leader at the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University. He is studying mechanisms of plant DNA replication and repair, as well as working on the development of new affinity chromatography systems for the purification of recombinant proteins.

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Separation of hydrophobic amino acid enantiomers in CEC system by molecular imprinting technique

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Capillary electrochromatography (CEC), first described by Pretorius, is a rapidly evolving hybrid technique between CE and LC. Analytes may be separated in CEC by the combined action of partitioning between stationary phase and mobile phase (chromatographic interactions) and by their difference in electrophoretic mobility (movement of solutes by electrical forces). Therefore, CEC usually possess higher efficiency and selectivity as compared with classical CE and LC. The use of CEC for chiral analysis has become popular in recent years due to high separation efficiency. Separation of enantiomers is very important because they may show different biological activities. Many single enantiomers of amino acids were also used for the synthesis of biologically important compounds. In this work, enantiomeric separation by CEC was extensively carried out using chiral monolithic capillary column. In this presentation, we will describe about the monolithic column, which is based on N-methacryloyl-(L)-phenylalanine methyl ester (MAPA) as hydrophobic monomer in CEC system.

Biography

Koray Şarkaya is PhD student from Hacettepe University and has done his PhD studies from Biochromatography and Biodiagnostics Research Group of Hacettepe University. He has been a Research Assistant at the Düzce University since 2009.

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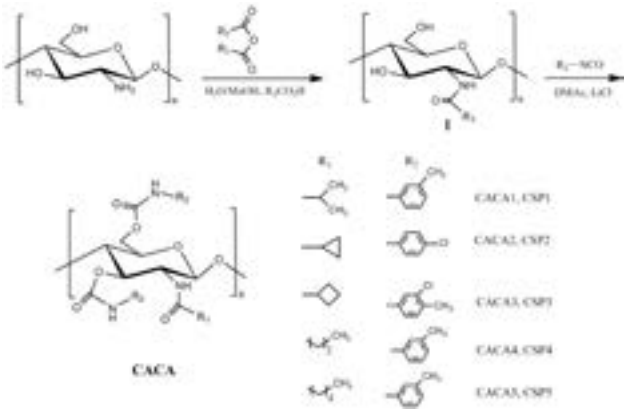
N-acylated chitosan Bis(arylcarbamate): A class of chiral separation materials with powerful enantioseparation ability and high tolerability to organic solvents

Sheng Tang, Jiande Liu, Qin Bin, Keqin Fu, Xiaochen Wang, Yingbin Luo and Zhengwu Bai
Wuhan Institute of Technology, China

Due to the fact that cellulose and amylose derivatives tend to dissolve in some organic solvents, the related polysaccharide derivatives-based CSPs prepared by coating manner can only work in a limited range of mobile phases, thus, restricting their widespread application in enantioseparation. Chitin, as one of the most abundant optically active biopolymers, is similar to cellulose in primary structure. Much lower solubility of chitosan derivatives was observed compared with the derivatives of cellulose and amylose, enabling the corresponding chitosan derived CSPs to be possibly worked in a wider range of mobile phases. Herein, in order to develop new types of chitosan derivatives-based CSPs, which not only are capable of excellent enantioseparation performance, but also bear a desirable tolerability, we introduce a class of coated-type CSPs, which are based on chitosan bis(arylcarbamate)-(amide) (CACA). The N-acylated chitosan derivatives were synthesized by the reaction of chitosan with carboxylic acid anhydrides in water/methanol in the presence of the corresponding carboxylic acids. CACAs were prepared by further derivatizing N-acylated chitosan with aryl isocyanates. Fig. 1 shows the structures of the prepared CACAs, which were then coated onto 3-aminopropyl silica gel, affording the corresponding CACAs-based CSPs. When the substituents introduced on acyl group at 2-position and on aryl group of phenylcarbamates at 3- and 6-positions were perfectly coordinated, the prepared N-acylated chitosan bis(arylcarbamate) would possess powerful chiral recognition and enatioseparation abilities, and meanwhile exhibited a desirable tolerability towards a wider range of mobile phases, consequently resulting in a new class of promising chiral separation materials.



The structures of the prepared CACAs.

Biography

Sheng Tang has completed his PhD in Analytical Chemistry from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. He serves as a teaching and research fellow at Wuhan Institute of Technology. He has published more than 10 papers in reputed journals.

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Permeation profiles of hormones and NSAIDs through vaginal mucosa using Pentravan cream

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Vaginal delivery is currently considered to be an important route for poorly-absorbed, rapid-metabolized oral drugs, and it also provides both local and systemic drug delivery. Drug absorption studies are compulsory to establish vaginal permeation kinetics, and in this work we evaluated the possibility of using Pentravan® (Fagron) as a vehicle to deliver drugs by this route. For this purpose, we used gestrinone, progesterone, testosterone, nimesulide and piroxicam creams using Franz diffusion cells with porcine vaginal mucosas. The vehicle was able to deliver approximately 88.17, 7.70, 22.87, 8.34, and 95.71 μg of gestrinone, progesterone, testosterone, nimesulide and piroxicam (respectively) per cm2 of skin by the end of the experiment, when considering only the drug that reached the receptor medium. We also evaluated resveratrol vaginal permeation. For that, we used a previously validated method and tested it with three different stationary phases: a commercial C18 column and two laboratory-made chromatographic columns containing poly(methyloctadecylsiloxane) (PMODS) thermally immobilized onto zirconized silica (Zr-PMODS) or titanized silica (Ti-PMODS). The transdermal vehicle used was also Pentravan®. The permeation experiments showed that resveratrol presented a high rate of retention within the vaginal mucosa, which suggests a local use rather a systemic one. This creates the hypothesis that the formulations with resveratrol, progesterone, nimesulide and piroxicam would be suitable for local vaginal treatments that could benefit from the diverse biological effects of these substances. We also highlight the potential of gestrinone and testosterone to act systemically when compounded using Pentravan®, making the route a viable alternative for other traditional routes.

Biography

Hudson Polonini has completed his PhD at 2014 from Federal University of Juiz de Fora. He studies analysis and control of medicines and related products, pharmaceutical and cosmeceutical technology, bio-pharmacy, natural products and (nano)ecotoxicology. He has published 40 papers in reputed journals, two patents and also some awards in innovation competitions.

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Automated harvesting and 2-step purification of eight 1-L unclarified mammalian cell-culture broths containing antibodies using a novel configuration on ÄKTA™ pure

Fabian Holenstein[1], Christer Eriksson[2], Ioana Erlandsson[2], Jill Simon[2], Lars Erni[2], Katharina Stein[3], Michael Glaettli[3], Andres Tschupp[1], Adriana Milicov[1], Patrick Schindler[1], Thomas Pietzonka[1], Johanna Koelln[1] and Jean-Marc Schlaeppi[1]

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Therapeutic antibodies represent one of the fastest growing segments in the pharmaceutical market. The growth of the segment has necessitated development of new efficient and cost saving platforms for the preparation and analysis of early candidates for faster and better antibody selection and characterization. We report a new integrated platform for unattended harvesting and 2-step purification of antibodies expressed transiently in HEK293T-cells at the 1-liter scale. The system consists of two bench-top preparative chromatography instruments connected to a central unit with eight disposable filtrations devices used for loading and filtering the crude biomass feeds. Our end-product QC analysis demonstrates that the quality of the material prepared by the 2-step automated purification is fully comparable to the material purified by standard manual 2-step purification. Average recoveries were also comparable to those obtained by manual purification, indicating that this automated system allows the cost-efficient preparation of therapeutic antibodies in the 20-200 mg range, and covers the requirements for early *in vitro* and *in vivo* profiling of drug candidates.

Biography

Fabian Holenstein holds an Engineering degree in Biotechnology and a Master's in Pharmaceutical Biotechnology. He works as a Senior Scientist in the section of protein production and antibodies at Novartis Institute for Biomedical Research in Basel/Switzerland.

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Romain Kapel et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Size-exclusion HPLC as a sensitive and calibrationless method for complex peptide mixtures quantification

Romain Kapel, Xavier Framboisier, Dominique Alonso, Ivan Marc and Alice Bodin
University of Lorraine, France

Nowadays, protein hydrolysates and fractions are of great interest because of their nutritional or bioactive properties. The quantification of total peptide concentration is of a crucial importance in order to establish mass balance of fractionation processes. This is commonly done either by Kjeldhal analysis, or by colorimetric assays, whose are laborious and time-consuming. This work describes an original methodology to quantify complex peptide mixtures by size-exclusion high-performance liquid chromatography (SE-HPLC), already used to characterize the molecular weight distribution of hydrolysate. In the proposed methodology, each point of the complex mixture chromatogram is regarded as a mixture of peptides sharing same molar extinction coefficient and molar weight, estimated from its retention time and the hydrolysate aminogram. This allows a conversion of absorbance into concentration (using Beer-Lambert law) and the integration of the overall signal gives the peptide concentration of the analysed fraction. The methodology was first tested on simulated elutions of peptide mixtures and a good estimation of the total peptide concentration was observed (error less than 10%). Then 30 fractions obtained by ultrafiltration of hydrolysates from two different sources were titrated by Kjeldahl or BCA analysis and analysed by SE-HPLC for an experimental validation of the methodology. Very good matchs between methods were obtained (error less than 15%). Moreover, the presence of organic solvents or salts in samples does not impact the accuracy of the methodology contrary to common quantification methods.

Biography

Romain Kapel is a third year PhD student from University of Lorraine, France. In 2013, she graduated as a chemical and process engineer. She is now working on the fractionation of protein hydrolysate during her PhD program. She will present an original methodology to quantify peptide concentration in complex hydrolysates by size-exclusion HPLC.

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Adéla Kotzianová et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Raman spectroscopy based method for the evaluation of compositional consistency of nanofibrous layers

Adéla Kotzianová[1,2], Jiří Řebíček[3], Ondřej Žídek[1], Marek Pokorný[3], Jan Hrbáč[2] and Vladimír Velebný[1,3]

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Electrospinning is currently a very popular method used across a number of industries. Electrospinning enables the production of nanofibrous layers of various structures and compositions. The production of a multi-component nanofibrous layer may result in an uneven distribution of the individual components throughout the layer. Confocal Raman spectroscopy combined with statistical methods allows these layers to be analyzed by determining their chemical composition and thus provides feedback for the spinning process. This paper presents a method which combines Raman spectroscopy analysis and its subsequent evaluation with singular value decomposition (SVD). Automated measurement of Raman spectra makes it possible to gather extensive spectral data from a particular area selected on a sample; the spectra are measured from a specific volume and not from individual fibers. Samples require no preparation for the analysis and the non-destructive nature of Raman spectroscopy ensures their reusability. When spectra of the individual component materials are included for reference, the subsequent SVD analysis of the spectral data makes it possible to determine the chemical composition of the measured areas, thus providing the content percentages of the individual components, which can be displayed either in the form of a scattered plot or a Raman map.

Biography

Adéla Kotzianová graduated from the Czech Technical University in Prague in 2012, where she earned her Master's degree in Instruments and Methods for Biomedicine. Currently, she is finishing her Doctoral studies in Physical Chemistry at the Masaryk University in Brno. Since 2012, she has been working as a researcher in the Department of Nanotechnology Device Development of the Contipro Holding. She has been specializing in spectroscopic methods since completion of her studies. In recent years, her work was focused especially on the preparation and analysis of nanofibrous materials. She has published 7 papers in reputed journals and presented her work on international conferences.

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Nguyen Khanh Hoang, J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Spectrophotometric determination of Cr(iii) and Pb(ii) using 5,11,17,23-tetra[(2-ethyl acetoethoxyphenyl) (azo) phenyl]calix[4]arene

Nguyen Khanh Hoang
Industrial University of Ho Chi Minh City, Vietnam

New complexes of 5, 11, 17, 23-tetra[(2-ethyl acetoethoxyphenyl)(azo)phenyl]calix[4]arene (TEAC) with Pb(II) and Cr(III) were prepared in basic solution with a mixture of MeOH and H_2O as solvent. The ratio of TEAC and metal ions complexation was found to be 1:1 under investigated condition. The complex formation constants (based on Benesi–Hildebrand method) for TEAC-Pb(II) and TEAC-Cr(III) were 4.03×10^4 and 1.2×10^4, respectively. Additionally, the molar extinction coefficients were 5×10^4 and 1.42×10^4 for TEAC-Pb(II) and TEAC-Cr(III), respectively. The H-point standard addition method (HPSAM) has been applied for simultaneous determination of complexes formation of Cr(III)/Pb(II) and TEAC with concentration from 2:1 to 1:20 (w/w). The proposed method was successfully utilized to invest lead and chromium content in plating wastewater samples. The results for several analyzed samples were found to be in satisfied agreement with those acquired by using the ICP-MS technique.

Biography

Le Van Tan obtained his PhD in Chemistry department in 1997 from Ha Noi National University, Vietnam. He completed his Post-doctoral in Seoul National University during 2004-2005 (Korea) and was Visiting Professor in Technical University Kaiserslautern during 2006, 2010 (Germany). He has published and presented 80 papers in International and National Conferences and journals. Currently, he is working as the Professor of this University and Professor of Chemical Engineering in Industrial University of Ho Chi Minh City. He is a member of Vietnam Chemical Society, Vietnam Analytical Chemical Society, ASC member and also Technical Committee for many international conferences on chemistry and chemical engineering in Japan, Singapore, Hong Kong, Thailand and so on. He is on Editorial Board of *Vietnam Journal of Chemistry*, Reviewer for the *International Journal of Chemical and Biomolecular Science* and many international journals on Chemistry. His interested research areas are finding new organic reagent for analytical chemistry; advanced separations and environment; postharvest technology.

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Notes:

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Inverse liquid chromatography in surface characteristic of materials

Katarzyna Adamska, Karol Kadlec and Adam Voelkel
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Examination of the physicochemical properties of different materials is important for their adsorptive or adhesive characteristics. Inverse Liquid Chromatography (ILC) technique differs from among generally known chromatographic methods, that it is not related to the separation of components of mixtures, but the interaction of test compounds with the investigated material, constituting the stationary phase of chromatography column. Interactions between the material and the test compounds affect the measured retention parameters and the shape of chromatographic peaks. Application of ILC seems to be useful to direct study of solid-liquid interactions in real conditions, which include: pressure, temperature and pH. This technique, in conjunction with proper mathematical models, allows evaluating the physicochemical characteristic of the biomaterials surface: its ability to various types of intermolecular interactions (e.g. capacity to donor-acceptor interactions), assessment of the impact of the number and type of functional groups on the surface activity. ILC can be also applied to characterize materials used in a separation process - mesoporous aluminosilicates (zeolites) for e.g. their ability to adsorption. The characteristic of the surface layer of such materials allows the analysis of the influence of the respective modifier's ligand for adsorption or separation processes. ILC technique thus allows the detailed characterization of the surface, taking into account its ability to various types of intermolecular interactions. This knowledge is essential for an appropriate development and improvement of different solid materials.

Biography

Katarzyna Adamska graduated at Wroclaw University of Technology at Faculty of Chemistry in 1999. In 2002, she started PhD studies at Poznan University of Technology in Institute of Chemical Technology and Engineering. In 2007, she got the academic degree of Doctor. Her PhD thesis entitled "Determination of the solubility parameter and its components for materials used in the pharmaceutical industry" was related, mainly, to the use of inverse gas chromatography in studies of different excipients, applied in pharmaceutical formulations.

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Tony Q Yan et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Enantiomer separations of amino acids and derivatives on immobilized polysaccharide phases with extended range of solvents using supercritical fluid chromatography

Tony Q Yan, Frank Riley, Laurence Philippe, Mark Hardink, Jared Van Haitsma and John Salisbury
Pfizer Inc., USA

This presentation discusses immobilized polysaccharide stationary phases with extended range of solvents for enantiomer resolution of select amino acids and derivatives using Supercritical Fluid Chromatography (SFC) and High Performance Liquid Chromatography (HPLC). Baseline resolution is achieved for selected amino acids and derivatives using this approach. The combination of an extended solvent (normally an aprotic solvent) in the presence of methanol and a suitable additive such as water, acid and base are critical for retention and resolution of amino acid pairs on immobilized columns. The amino acid separations are also achieved on the coated polysaccharide phases with alcohol based solvents in the presence of acid and base. In addition, the use of cyclofructan and polysaccharide phases for amino acids and small peptides separations along with the use of reversed phase chiral stationary phase for very polar compounds, acids and diacids, are also discussed.

Biography

Tony Q Yan is currently working for Pfizer, Inc. (Groton, CT, USA) in the field of impurity isolation for structure elucidation in the Department of Pharmaceutical Science. He has been working in pharmaceutical research and development in the area of chiral and achiral purifications, and impurity isolation for over 20 years since he graduated from the Department of Chemistry in University of Missouri in Rolla with PhD degree in 1995.

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Aschwin van der Horst et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Comprehensive two-dimensional critical chromatography a tool to understand the behavior of random and block copolymers in chromatography

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Random, gradient and block copolymers are behaving differently in commonly used solvents in liquid chromatography. Copolymers show two or more distributions, depending on their topology. Linear polymers and random copolymers can be fully characterized by combining their chemical-composition distributions (CCD) and molecular-weight distributions (MWD); block copolymers can be characterized by their block-length-distribution (BLD). Liquid chromatography at the critical point of adsorption (LC-CC or simply critical chromatography, CC) is a highly useful technique used in polymer LC. The mechanism of CC can be studied by coupling LC with size-exclusion chromatography (SEC) yielding CCxSEC. This technique is often used for the characterization of functionality-type-distribution (FTD) in combination with MWD. What we would like to demonstrate is another approach; two-dimensional critical chromatography (CCxCC). Using this approach, more insight in the composition of random-, gradient- and di-block copolymers is obtained. Also the possibility of obtaining relevant information from the CCxCC analysis of the latter copolymers is investigated. As a step towards a complete understanding of the behavior of block copolymers in liquid chromatography, we are showing the results of a comprehensive method for determining the mutually dependent block-length distributions of the blocks in di-block copolymers. When critical chromatography is used in two-dimensions of a two-dimensional liquid chromatography (LCxLC) set-up, all relevant distributions (the molecular-weight, chemical-composition and block-length distributions (CCD, BLD(A), BLD (B), MWD, conversion and degree of gradient distribution (DGD)) can be determined in one analysis.

Biography

Aschwin van der Horst is a Principal Chemist Analytics in the Analysis, Materials and Instrumentation Laboratory at Nuplex, Bergen op Zoom, Netherlands. He holds an experience of 16 years as a Polymer Analytical Chemist and 11 years of experience in Coating Technology. He published various papers on the analysis and characterization of polymers by Multidimensional Liquid Chromatography and Pyrolysis-Liquid Chromatography. Besides his work at Nuplex, he is also a Guest Lecturer at the Avans Hogeschool Breda on Polymer-, Resin- and Paint Analysis and its characterization.

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Michael John et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
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Chromatography in the NMR tube

Michael John, Thomas Niklas, Jannis Gottwald and Dietmar Stalke
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Recent developments in nuclecar magnetic resonance (NMR) equipment permit the spatially resolved observation of NMR signals on routine instruments that are part of virtually every chemistry laboratory. Specifically, a series of thin (~ 1 mm) horizontal slices may be excited and recorded of the sample instead of the ~20 mm bulk volume within the rf coil. Through referencing with solutions of known concentrations, local concentrations can be determined. We have recently applied slice-selective NMR spectroscopy to a range of chemical problems: A first example was the monitoring of the anisotropic swelling of cross-linked polymers in organic solvents and determination of the homogeneity of anisotropy across the polymer. Further applications were a "single-shot" NMR titration, where the signal of the first component was resolved over a concentration gradient of the "titrated" component, and a reaction monitoring, where two reagents diffuse towards each other in the sample tube. Here, we present another application: “chromatography in the NMR tube” (a glass tube with Ø = 5 mm): Two compounds that may differ in molecular size or polarity diffuse downwards through a polymeric matrix (which may be cross-linked polystyrene or even silicone grease) at their individual rates and appear in the slices at the bottom with individual concentrations. Although no complete separation of the compounds is achieved, signal assignment is facilitated, and diffusion coefficients may be calculated as an alternative to the DOSY method.

Biography

Michael John completed his PhD with Horst Kessler at the Technical University of Munich in 2004, and then spent 2 years with Gottfried Otting at the Australian National University, Canberra. Since 2007, he is Lecturer and Director of the NMR facility at the University of Göttingen. His list of publications includes more than 60 papers in peer-reviewed journals and 25 conference contributions.

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Notes:

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Aptamer-based affinity chromatography as a rapid, single step method for purification of native proteins

Svetlana M Krylova, J Bao, S Boloborodov, O Borisade and S N Krylov
York University, Canada

Isolation and purification of recombinant proteins is one of major tasks of modern biotechnology. Isolation of enzymes and antibodies requires conditions that could preserve biological activities of proteins. Often fusion of proteins with His-, GST-, and MBP-tags is used to facilitate their isolation by affinity chromatography. However, the tags, may interfere with the application of the protein while there removal is often accompanied by protein's loosing its biological activity. We developed aptamer-based affinity chromatography allowing isolation of the recombinant proteins from the crude cell lysate as a quick method yielding native biologically active enzymes. DNA aptamers to AlB protein were developed and characterized by Kinetic Capillary Electrophoresis (KCE). Synthetic DNA aptamers with K_d values in the nanomolar range were used for selective binding and isolation of AlkB from the cell lysate. Specifically, gold (DE3) bacterial culture of cells, expressing E. coli AlkB protein was loaded on aptamer-modified magnetic beads (immobilized though a biotin-streptavidin link). The unwanted components of the cell lysate were removed by washing the beads. AlkB was eluted using different solutions with high ionic strengths. The results were compared with the activity and yield of the enzyme purified using standard tag-based methods of protein purification. Our new method was also succesfully repeated for isolation and purification of MutS protein. In my presentation, I will discuss the CE based aptamer development technology, and I will demonstrate the potential of using aptamers for purification of enzymes from cell lysates in a single simple step, providing biologically active pure recombinant proteins.

Biography

Svetlana M Krylova completed her PhD from the Russian Academy of Sciences. She has over 10 years of research leadership experience in the area of Medical Diagnostics and Drug Development in biotechnology and pharmaceutical companies in Canada. She has been a contract faculty member at York University in Toronto since 2008. She is leading research projects in the area of Bioanalytical Chemistry as a Senior Research Associate in the Centre for Research on Biomolecular Interactions at York University.

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Notes:

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Removal of lipid interferences using zirconia-based Solid Phase Extraction (SPE) and QuEChERS sorbents

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[1]MilliporeSigma, USA
[2]Sigma-Aldrich Chemie GmbH, Germany

Although lipids are essential to life processes, they pose an obstacle in both bioanalysis and food analysis. In addition to producing inaccurate detection limits and contaminating chromatographic systems, interfering lipids can ultimately lead to the shortened lifespan of columns and instruments. Traditional solid phase extraction (SPE) cleanup techniques often provide insufficient lipid matrix removal. Therefore, the use of zirconia-based sorbents has been developed for selective lipid removal, and consequently, better analyte determination. In addition to hydrophobic interactions, zirconia-based sorbents utilize Lewis acid/base interactions to selectively retain undesirable lipid interferences. In SPE and/or dispersive SPE (QuEChERS) formats, these zirconia sorbents may be combined with traditional phases like C18 to further improve inference removal. Compared to traditional cleanup sorbents, these innovative sorbents have been shown to remove more lipid matrix interferences, including di, tri-, monoglycerides and phospholipids. A comparison of zirconia-based sorbents to traditional cleanup sorbents for lipid removal from various food and biological matrices will be demonstrated. Background removal, analyte recovery, and reproducibility of the different cleanup techniques will be compared in this presentation.

Biography

Jennifer E Claus has graduated from Lycoming College in 2001 with a Bachelor of Science in Chemistry and Biology. She has spent six years working at Merck & Co. in Rahway, NJ as a Medicinal Chemist. While at Merck, she attended Rutgers University and completed her Master of Science in Chemistry. She has been with MilliporeSigma for the past nine years, initially working as a Chiral Applications Chemist for the first four years of her MilliporeSigma career. She has been in her current marketing position as a Product Manager for Sample Preparation, for the past four years.

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