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Chapter 1 : Publications | Limbach Group Biological Mass Spectrometry | Patrick A Limbach

*Separation and identification of oligonucleotides by hydrophilic interaction liquid chromatography (HILIC) - inductively coupled plasma mass spectrometry (ICPMS) Renee N. Easter, 1 Karolin K. KrÃ¶ning, 1 Joseph A. Caruso, 1, * and Patrick A. Limbach 2, *.*

Abstract A method for the selective detection and quantification of peptide: Mobile phase conditions that allow separation of heteroconjugates while maintaining ICPMS compatibility were investigated. Inductively coupled plasma mass spectrometry, Protein-nucleic acid cross-links, Elemental phosphorus detection, Liquid chromatography-mass spectrometry, Quantitative analysis Introduction Protein: Chemical and photochemical cross-linking has proven to be a useful approach for covalently linking proteins with nucleic acids to impose distance constraints on such complexes [7 - 13]. These constraints, when combined with data from biophysical techniques such as X-ray crystallography, nuclear magnetic resonance NMR , and cryoelectron microscopy, allow researchers to develop more accurate three-dimensional structures, thereby improving our understanding of these biologically significant complexes [14 - 17]. While a number of biophysical approaches have been used for characterizing protein: The potential advantages of MS for the analysis of cross-links include its ability to analyze high molecular weight complexes either intact or through analysis of enzymatic digestion products, the relatively high sensitivity of MS for peptides and oligonucleotides, and the compatibility of this method with a variety of different cross-linking reagents [8 , 12 , 19]. Although MS methods are especially useful in identifying the proteins involved in protein: A large number of analytical developments to improve the sensitivity and reliability of cross-link detection have been pursued, primarily by Urlaub and co-workers [8 , 11 , 13 , 21 - 23], along with some work by our group [24 - 26]. Most recently, the use of electron-induced dissociation methods for complete sequencing of electrosprayed peptide: This discovery was surprising as ICPMS is a powerful analytical tool for element-specific analysis, as the signal response is directly proportional to the element concentration in the sample and this ionization source is not matrix or sequence dependent [28 , 29]. Recent ICPMS developments allow for the detection of previously intractable elements such as phosphorus. Quantitation of post-translationally modified proteins phosphoproteins and sulfated proteins by phosphorus or sulfur monitoring during LC-ICPMS analysis of proteolytic digests is now commonly performed [28 - 31]. Similarly, LC-ICPMS monitoring of phosphorus or sulfur can also be used for the identification and quantification of oligodeoxynucleotides and phosphorothioate oligonucleotides [32 , 33]. This work is based on the premise that such heteroconjugates can be selectively detected by monitoring for elemental phosphorus, which is present in the heteroconjugate from the phosphodiester linkage of the oligonucleotide, using ICPMS. If possible on these heteroconjugates, this instrumental approach could then enable the selective detection and quantification of protein: Before pursuing such cross-links, these initial studies have focused on the appropriate chromatographic and instrumental conditions required to detect phosphorus arising from the oligonucleotide component of the heteroconjugate, and to identify the analytical figures of merit for quantitative analysis of phosphorus from such heteroconjugates. Multiple chromatographic conditions were investigated, and a suitable analytical method for characterizing heteroconjugates is demonstrated using a model peptide: PEEK fused silica tubing. An Agilent Zorbax Extend C18 capillary column 0. The TFA-based mobile phase consisted of mobile phase A of water with 0. Interference removal is accomplished by the ORS through kinetic energy discrimination. The polyatomic spectral interferences are eliminated when pressurized gas fills the cell and collides or reacts with such molecules resulting in a loss of kinetic energy. Instrumental parameters used were RF forward power, , plasma Ar gas flow rate, ICPMS enables element-specific detection e. As the ionization step in ICPMS first requires vaporization and atomization of the sample solution, the use of high amounts of organic solvents can lead to plasma instability and carbon deposition on the interface cones. Because the separation of biomolecules, such as peptides and oligonucleotides, using RP-HPLC typically requires high concentrations of

organic solvent or buffer to elute the sample from the column, care must be taken to reduce the overall volume of LC eluent introduced into the ICP source. The acidic mobile phases and solution conditions investigated have been utilized previously with heteroconjugates, such as that used here as a model system, and were not found to affect the integrity of the heteroconjugate [13 , 21 , 25 , 26]. To initially investigate these conditions, several standard peptides including bradykinin, ACTH and neurotensin, along with a tryptic digest of bovine serum albumin and an oligodeoxynucleotide dT10 were used as representative samples. Not surprisingly, under these HPLC conditions, all of the peptides investigated could be retained on the column while the oligodeoxynucleotide eluted in the void volume data not shown. Similarly, when these same samples were analyzed using HPLC mobile phases typically used for oligonucleotide separation, only the oligodeoxynucleotide was significantly retained on the same C18 column data not shown. Consistent with previous results from LC-ESI-MS [25], the nature of the heteroconjugate will dictate the optimal chromatographic conditions. Peptide-like heteroconjugates should be retained on the stationary phase effectively under these conditions. Phosphorus Detection and Matrix Effects While the initial investigations into HPLC conditions were conducted to verify the applicability of such conditions for samples containing peptide- and oligonucleotide-like components, detection was performed using UV spectroscopy. The next series of experiments focused on the applicability of these mobile phases using ICPMS for element-specific detection of heteroconjugates. Phosphorus is present from the oligonucleotide component of the heteroconjugate and, except when phosphopeptides are present, background interferences are expected to be minimal, provided the ORS is used. LC-ICPMS conditions were first optimized using Pp60, a phosphorylated peptide, as the model compound for peptide-like HPLC conditions and dT10 as the model compound for oligonucleotide-like HPLC conditions, after which the effects of background non-phosphorus-containing species on the identification of these model compounds was pursued. A tryptic digest of BSA was chosen as a representative sample matrix to examine whether a background of non-phosphorylated peptides would interfere with the detection of phosphorus-containing samples, such as a heteroconjugate, during ICPMS analysis. The interest here was to identify whether heteroconjugates would need to be purified extensively from uncross-linked protein samples prior to LC-ICPMS. By way of comparison, the UV chromatogram detection at nm of the same run Figure 1b reveals numerous eluting peaks arising from the tryptic peptides from BSA as well as the spiked Pp

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Renee N. Easter's 12 research works with 90 citations and reads, including: Separation and identification of phosphorothioate oligonucleotides by HILIC-ESIMS. Renee N. Easter has expertise in.

The application of elemental tags for biological analyte identification PhD, University of Cincinnati, , Arts and Sciences: Chemistry Metallomics is the study of the metallome, interactions, and the functional connections of metal ions and other metal species with genes, proteins, metabolites, and other biomolecules in biological systems. It has been applied to a variety of samples from biological to environmental. Elemental analysis along with elemental and molecular speciation is a major component of metallomics experiments. Molecular speciation analysis is performed best with high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry ICPMS in conjunction with molecular mass spectrometric techniques. A wide range of liquid chromatography techniques including capillary and conventional to hydrophilic interaction liquid chromatography to strong cation exchange chromatography can be applied for the separation of biological samples. ICPMS is the gold standard for elemental analysis at trace levels ppt for most elements and it is known for its sensitivity and selectivity. Liquid chromatography can also be combined with molecular mass spectrometry as well to offer excellent molecular identification of biomolecules. The first part of this dissertation focuses on novel ways to separate and identify phosphorothioated oligonucleotides. Both methods offer low detection limits and a fast and easy way for identification. The second part of this dissertation focuses on metallomics and proteomics techniques for the analysis of a more complex matrix of cerebral spinal fluid CSF. Subarachnoid hemorrhage SAH and its sequela cerebral vasospasm CV , kill or seriously debilitate an estimated 1. The ability of these techniques to help identify biomarkers has the potential to save lives but no biomarkers for CV have been identified yet. The focus of the first project was to explore CSF for selenoproteins that could play a role in the etiology of CV. Both elemental and molecular along with multidimensional separation techniques were applied for this exploration. The focus of the second project was to identify proteins that had significant differences between three samples types with common proteomics techniques that include multidimensional separation techniques and two different molecular mass spectrometric techniques. Typically, gangliosides are part of the ganglion, a group of nerve cell bodies where they act as neuroprotective agents, support, and maintenance of the mature neuronal cells. Glaucoma is an age-related neurodegenerative disorder of the eye that leads to blindness. Retinal ganglion cells RGCs in retinal tissue of the eye, degeneration is seen followed by the optic nerve head damage. From immunohistochemistry studies with cholera toxin-B, gangliosides which are part of the RGCs were lost during the RGCs degeneration. An optimized ganglioside isolation technique was developed which is a combination of liquid-liquid extraction for lipid phase separation followed by a solid-phase extraction for desalting and removal of debris. The optic nerve studies using electron microscopy was undertaken to establish the glaucoma disease progression. Electron microscopy study results show no change in the optic nerve count which represents no glaucoma in old age animal samples. Thus, a non-correlation was seen with the ganglioside changes compared to optic nerve counts. A greater animal sample with much older age provides a better understanding of gangliosides role in glaucoma. Koch, Jennifer C Analysis of FruHis, a potential bioactive Amadori compound in processed tomatoes Master of Science, The Ohio State University, , Food Science and Technology Epidemiological evidence indicates that diets rich in tomatoes are associated with a reduced risk of chronic diseases and certain cancers, particularly prostate cancer. The carotenoid pigment lycopene has received the most attention as a bioactive compound in reducing the risk of prostate cancer, but a causal relationship has not yet been established. Consumption of processed tomato products has been associated with an even lower risk of prostate cancer than fresh tomatoes, indicating the potential importance of processing-induced compounds in cancer prevention. The Maillard reaction occurs during food processing in tomatoes and other foods and may be a source of bioactive compounds. Amadori compounds are formed as

an intermediate in this reaction and their production is affected by temperature, time, pH, and water activity during processing. One particular Amadori compound, fructose-histidine, or FruHis, was recently shown to work in concert with lycopene in processed tomatoes in vitro and in vivo to further reduce the risk of prostate cancer in animal studies. However, very little is known about the formation of FruHis and other Amadori compounds during food processing. In addition, comprehensive methods for the separation, identification, and quantification of FruHis and Amadori compounds are lacking. A HILIC method using formic acid and acetonitrile was developed on an amide column to successfully separate these compounds and was particularly suited for quantification of FruHis. This method was used to quantify FruHis during typical tomato paste processing. The FruHis content increased during tomato paste concentration, reaching a maximum of 3. The initial formation of FruHis was tied to a key combination of heat applied and water lost as measured by percent soluble solids. The content of FruHis in a number of commercial juices, sauces, pastes, and powders ranged from 1. The results of this work will provide a basis for future studies on the biological activity of FruHis and Amadori compounds and may one day lead to the development of a FruHis-rich tomato-based functional food for cancer prevention.

Chapter 3 : Patrick A. Limbach - Publications

*Separation and identification of oligonucleotides by hydrophilic interaction liquid chromatography (HILIC)â€" inductively coupled plasma mass spectrometry (ICPMS) Renee N. Easter, a Karolin K. KrÃ¶ning, a Joseph A. Caruso * a and Patrick A. Limbach * b.*

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sweeping with a cationic surfactant in microchip capillary electrophoresis. Investigation of single-site zirconium azaborolinyl complexes by laser desorption ionization time of flight mass spectrometry. Developing limited proteolysis and mass spectrometry for the characterization of ribosome topography. Characterization and performance of injection molded poly methylmethacrylate microchips for capillary electrophoresis. Study of injection bias in a simple hydrodynamic injection in microchip CE. Frontal analysis in microchip CE: Mass spectrometry-based detection of transfer RNAs by their signature endonuclease digestion products. Bisphenol A facilitates bypass of androgen ablation therapy in prostate cancer. Characterizing the reproducibility of a protein profiling method for the analysis of mouse bronchoalveolar lavage fluid. Journal of Proteome Research. Unlimited-volume electrokinetic stacking injection in sweeping capillary electrophoresis using a cationic surfactant. On-line sample preconcentration by sweeping with dodecyltrimethylammonium bromide in capillary zone electrophoresis. Mass spectrometry of RNA: On-line sample preconcentration using field-amplified stacking injection in microchip capillary electrophoresis. Effects of streptomycin resistance mutations on posttranslational modification of ribosomal protein S Analytical performance of polymer-based microfluidic devices fabricated by computer numerical controlled machining. The autofluorescence of plastic materials and chips measured under laser irradiation. Lab On a Chip. Association of the kDa extrinsic protein with photosystem II in higher plants. Estimation of pK a values using microchip capillary electrophoresis and indirect fluorescence detection. Shotgun sequencing small oligonucleotides by nozzle-skimmer dissociation and electrospray ionization mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. Quantitation of ribonucleic acids using 18O labeling and mass spectrometry. Photofunctionalization of polymer microfluidic devices for mass spectrometry Aiche Annual Meeting, Conference Proceedings. Development of polymeric microchips for use in binding studies Aiche Annual Meeting, Conference Proceedings. Thermus thermophilus L11 methyltransferase, PrmA, is dispensable for growth and preferentially modifies free ribosomal protein L11 prior to ribosome assembly. The use of mass spectrometry in genomics. Bacterial biofilms of importance to medicine and bioterrorism: Expert Opinion On Biological Therapy. Covalent immobilization of proteases and nucleases to poly methylmethacrylate. Integrating micromachined devices with modern mass spectrometry. Development of an integrated polymer-based microfabricated device for the mass spectrometric characterization of proteins Proceedings 50th Asms Conference On Mass Spectrometry and Allied Topics. Interfacing a polymer-based micromachined device to a nanoelectrospray ionization fourier transform ion cyclotron resonance mass spectrometer Analytical Chemistry. Automated, web-based, second-chance homework Journal of Chemical Education. Mononucleotide gas-phase proton affinities as determined by the kinetic method Journal of the American Society For Mass Spectrometry. A comparison of charge-transfer and traditional maldi matrices for the mass spectrometric analysis of polymers American Chemical Society, Polymer Preprints, Division of Polymer Chemistry. Electrospray ionization mass spectrometry of metalloporphyrins Journal of Mass Spectrometry. Matrix-assisted laser desorption-lonization mass spectrometry: An overview Spectroscopy Santa Monica. Indirect mass spectrometric methods for characterizing and sequencing oligonucleotides Mass Spectrometry Reviews. Molecular mass measurement of intact ribonucleic acids via electrospray ionization quadrupole mass spectrometry. Ion trajectories in an electrostatic ion guide for external ion source fourier transform ion cyclotron resonance mass spectrometry. An anticodon sequence mutant of Escherichia coli initiator tRNA:

Chapter 4 : OhioLINK ETD: Easter, Renee N.

Specifically, the evaluation of highly polar compounds (i.e., compounds that cannot be retained on traditional reversed-phase stationary phases) has been challenging, and a hydrophilic interaction chromatographyâˆ'electrospray ionization mass spectrometry (HILICâˆ'ESI-MS) method was developed to meet this need.

Chapter 5 : Routledge Handbooks Online

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Renee Easter, Colin Barry, Joseph Caruso, Patrick Limbach Analytical Methods , Evaluation of core-shell particle columns for ion-pair reversed-phase liquid chromatography analysis of oligonucleotides.

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Handbook of analysis of oligonucleotides and related products. Analysis by hydrophilic interaction chromatography (HILIC) / Patrick Limbach and Renee N. Easter.

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As you are new to chromatography, please take the time to first read many of the papers, articles and books which describe these fundamental HPLC relationships (esp for NP/RP modes).