P-M-0403 **Revealing the Ways of Manipulating Selectivity of Covalently-bonded Anion Exchangers for Ion Chromatography Toward Mono- and Polyvalent Organic Acids.** Aleksandra Zatirakha, Anna Uzhel, Anastasia Borodina, Igor Kvachenok, Alexander Smolenkov, Oleg Shpigun, Lomonosov Moscow State University, Moscow, RUSSIA

Determining organic acid profiles in presence of inorganic anions is one of the key analytical tasks in food and beverage industry, which allows one to control the quality and authenticity of the products. Some common chromatographic techniques for organic acid analysis suffer from either limited resolution and impossibility of simultaneous determination of organic and inorganic anions (ion-exclusion chromatography, ICE), high costs (ICE-MS), incompatibility with suppressed conductivity detection (mixed-mode liquid chromatography), or lack of sensitivity (capillary electrophoresis). In contrast, suppressed ion chromatography (IC) is a sensitive, reliable, simple, and relatively inexpensive method, which makes it an attractive technique for solving the above-mentioned task. Nowadays, there are a lot of selective stationary phases for IC on the market that are suitable for separating multi-component mixtures of organic and inorganic anions, but their common problem is insufficient resolution of some low molecular-weight organic acids and divalent species, which are of interest not only in food and beverage, but also in power plant and pharmaceutical industries. Chemically-derivatized anion exchangers with covalently bonded polyamines or hyperbranched layers proved to be promising materials for the separation of complex mixtures of inorganic anions and organic acids. In this work, after testing 20 anion exchangers, we discovered several ways of manipulating selectivity of such phases to provide improved resolution of short-chain carboxylic acids and some polyvalent species. It turned out that the factors providing the most significant selectivity variations are the branching degree and the hydrophilicity of the functional ion-exchange layer, the structure of the amine in the outer part of the layer, the type of quaternizing agent for such amine, and also the presence of polar and negatively charged substitutes closer to the substrate surface. Very interesting trends were revealed after studying the effect of temperature on the separation of weakly retained organic acids for several types of covalently-bonded stationary phases, which provided the possibility to significantly improve the resolution for certain pairs of tested analytes. Combining the optimized functional layer structure and separation conditions, we managed to prepare the first anion exchangers for suppressed IC, which provide simultaneous determination of the whole set of low molecular-weight organic acids (quinic, gluconic, shikimic, glycolic, acetic, formic, lactic, galacturonic, and propionic), seven standard inorganic anions, oxyhalides, and some polyvalent organic acids. Several anion exchangers providing the separation of up to 27 anions were successfully used for the analysis of beverages (juice, wine, beer), pharmaceutical preparations, and environmental samples.

P-M-0404 **Investigating the Retention Mechanisms and Types of Secondary Interactions Determining the Influence of Structural Fragments of Novel HILIC Materials on Their Selectivity.** Alla Chernobrovkina, Aleksandra Zatirakha, Alexander Popov, Ilya Kovalenko, Alexander Smolenkov, Oleg Shpigun, Lomonosov Moscow State University, Moscow, RUSSIA

Since the selectivity of HILIC stationary phases is crucial for choosing a stationary phase for separating target compounds, it's important to find out the particular effect of a certain functional group or a linker on the chromatographic performance of the material. Revealing the respective trends can help to develop the ways of regulating the interactions of the stationary phase with target analytes and constructing novel materials with improved selectivity toward the particular classes of polar compounds. One of the most representative methods for versatile comparison of stationary phases is an examination procedure for HILIC materials known as Tanaka test, which allows one to explore different types of selectivity and thus to observe specific secondary interactions of analytes with an adsorbent. In the present work a step-by-step construction of novel HILIC stationary phases was performed with consistent variations in their functionalities using different linkers, functional groups of diverse nature such as amines, polyamines of different hydrophilicity, diol-, zwitterionic groups differing in their structure and acidity, as well as variations in grafted polymer layer structure by means of its hydrophilization, quaternization, and crosslinking. Tanaka tests were used to characterize the whole set of home-made HILIC materials in addition to the study of their retention characteristics obtained for model mixtures of various analytes (sugars, water-soluble vitamins, and amino acids, including positively charged arginine, histidine, lysine). Synthesized phases were characterized in terms of selectivity for methylene and hydroxyl groups, configurational isomers, ion-exchange interactions, and their acidic-basic nature. All adsorbents were also tested for re-equilibration time to establish their tolerance to gradient elution mode. The obtained results allowed us to reveal, which particular changes in the structure of the HILIC phases (and to which extent) are responsible for their differences in chromatographic performance. In particular, the type of surface coverage leading to the increase in hydrophilicity was established. Comparison of ion-exchange properties of the synthesized phases revealed unexpected ion-exchange selectivity in some cases for the phases that tended to have neutral surface. It allowed us to find the ways of regulating their cation- and anion-exchange properties caused by the certain fragments in the structure responsible for ion-exchange mechanism. Tanaka tests

provided the possibility to classify the set of 15 home-made HILIC phases and reveal impacts of certain structural fragments of the material on its selectivity, which could be useful for developing the ways of constructing multifunctional HILIC stationary phases selective for broader range of analytes.

P-M-0405 **Evaluation of Two Hydrophilic Interaction Liquid Chromatography Stationary Phases for Global Metabolomics Analysis of Human Plasma.** Rosalynde Sonnenberg, Dajana Vuckovic, Concordia University, Montreal, CANADA

To ensure coverage of as many metabolites as possible, from hydrophobic to hydrophilic, both reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) are used. A variety of stationary phases are available for HILIC and can be grouped into three categories: neutral, charged, and zwitterionic. Neutral stationary phases often contain amides or diols, charged stationary phases often contain amino groups or silica, and zwitterionic stationary phases often contain sulfobetaine or phosphorylchlorine groups. Each stationary phase chemistry has varying relative hydrophobicity, and different hydrogen bonding and electrostatic interaction capabilities. Contrepois et al. previously reported that, of five stationary phase chemistries tested (BEH amide, BEH HILIC, Hypersil Gold HILIC, Synchronic HILIC, and ZIC-HILIC), the ZIC-HILIC column with a neutral pH mobile phase provided the best results for global metabolomics of human plasma. The objective of this study was to compare this zwitterionic ZIC-HILIC method to the HILIC method using charged HILIC stationary phase, specifically Ascentis Si Express stationary phase. During method development, the salt concentration in the mobile phase and the mobile phase gradient were both investigated. The quality of the peak shapes, peak separation capability, metabolite coverage, and method robustness were used to compare the results from each stationary phase. The methods were initially evaluated using a mixture of 31 standards covering a range of logP values (-4.9 to 2.26), molecular weights (103 to 776 Da), and multiple classes of metabolites including but not limited to vitamins, amino acids, hormones, and neurotransmitters. Good quality results for 15 and 17 of the metabolite standards were achieved using the silica and ZIC-HILIC columns, respectively. No peaks were observed for 10 and 5 of the standards on the two phases respectively. Although the ZIC-HILIC column provided observable results for five more standards than the silica method, 4 of which were of good quality, two metabolites were also seen using the silica method that were not seen with the ZIC-HILIC method, showing a certain degree of complementarity between the two methods. The optimized methods were finally compared using a complex sample: methanol precipitated plasma.

P-M-0406 **High Performance Separations using 100% Aqueous Mobile Phase Compatible Superficially Porous Particle Columns Coupled with Mass Spectrometry.** Chuping Luo, Justin Godinho, Benjamin Libert, Stephanie Schuster, Barry Boyes, Advanced Materials Technology, Wilmington, DE, USA

A challenge for analysis of highly polar compounds by HPLC is low retention on reversed-phase columns. To increase retention, low concentration of organic solvents is used, potentially at the expense of phase stability, referred to as dewetting. The HALO AQ-C18 is a superficially porous particle packing material specifically designed to address stability under highly aqueous conditions, while maintaining high retention and high separation efficiency of polar compounds. One such class of polar compounds that have been investigated in detail are the purines and their metabolites. An example studied in detail includes the enzyme guanine deaminase (GD). GD catalyzes purine catabolism of guanine into xanthine and ammonia. GD levels are high in telencephalic regions and low in white matter and cerebellum tissues in mammalian brain. The significance of this expression pattern on synaptic physiology is uncertain. Here, we present a sensitive and selective LC/MS assay for GD to improve understanding of the pharmacology of this enzyme. Using 100% aqueous conditions and no ion pairing reagents, the column exhibited no evidence of phase collapse typical of traditional C18 sorbents. Highly reproducible analyses in addition to robust wash methods were conducted in less than 4 minutes. This allowed for rapid screening and replicate enzymatic assays run in parallel. This stationary phase affords high throughput and reproducible separations in 100% aqueous mobile phases and is demonstrated in combination with highly selective and sensitive mass spectrometry and a range of highly polar analytes.

P-M-0407 **Exosome Isolation from Cell Culture Milieu by HIC on Polyester Capillary-channeled Polymer Fiber Phase.** Sisi Huang, R. Kenneth Marcus, Clemson University, Clemson, SC, USA

Exosomes are membranous vesicles (30-100 nm) secreted by most cells, which play a key role in intercellular communication, transportation of genetic information and tumorigenic proteins, mRNA and miRNA. Exosomes hold a great deal of promise in disease diagnostics, as they display the same protein biomarkers as their originating cell. A major limitation of exosome study is the hardship and the lack of standardization for already challenging techniques to isolate exosomes. Current exosome isolation methods have practical problems including being too time-consuming and inefficient, destructive to exosomes, or too costly for use in a clinical setting. This study developed an efficient, low-cost and mild