K6 Applications of Hydrophilic-Interaction Chromatography in Forensic Science

Thomas A. Brettell, PhD, and James N. Anasti, BS, Cedar Crest College, 100 College Dr, Allentown, PA 18104*

After attending this presentation, attendees will have a better understanding about hydrophilic interaction liquid chromatography and review of literature displaying how this method can be used for the forensic science community.

This presentation will impact the forensic science community by providing information on the application of Hydrophilic Interaction Liquid Chromatography (HILIC) to the separation of analytes in different matrices including forensic drug and toxicological samples.

HILIC is a mixed or multi-modal partition chromatography designed specifically to separate polar, ionic, or weakly acidic and basic compounds. The aqueous/organic mobile phase is passed over the more polar stationary phase. Columns consist mainly of bare silica or chemically bonded silica such as simple amide, cyano, and diol to complex alkyl and polymeric coatings. The columns can be particle packed or monolithic. The bonded moieties can range in thickness to allow for specific aqueous saturation. The aqueous layer creates electrostatic repulsion and other intermolecular forces to aid in the separation process of more similar compounds such as isomers.

The highly organic mobile phase is composed mainly of acetonitrile and can be controlled through gradient or simple isocratic elution. High concentrations of organic modifiers allow for proper ionization of analytes and, therefore, are compatible with an electrospray ion source of a mass spectrometer. Due to advances in mass spectrometry, this is the detector type of choice when looking at low concentration of analytes and analytes in difficult matrices such as whole blood. As an alternative to normal and reverse phase liquid chromatography, HILIC sustains selectivity and prominent peak shape while using rapid isocratic methods.

Reversed reverse-phase or aqueous normal phase chromatography was coined HILIC by A.J. Alpert in 1990. The first applications of HILIC were primarily of bio analytics such as proteomics and metabolomics because of the ability to purify bio markers, amino acids, and other proteins. In the pharmaceutical industry, the use of HILIC has grown for purposes such as quality control and processes pertaining to research and development. Although HILIC is not a new technique, this form of chromatography is beginning to become more prevalent because of stationary phase developments. Advances in stationary phase preparation, including nanostructures within polymeric scaffold, create efficient preparation and productive permeability. The new production techniques allow not only for a variety of moieties, but also lower cost and create a more consistent product. The separation efficiencies and increased production of HILIC columns has significantly amplified research and applications. Today, HILIC can be seen to be applied to broader applications. HILIC has been applied to many fields, including forensic science and forensic toxicology. Designed for polar metabolites, HILIC is a valuable asset to forensic toxicologists for the analysis of such polar drug metabolites.

Reviews of forensic HILIC applications are seen in such studies as comparison of ethyl-glucuronide distribution in pubic and head hair. Other topics include body fluid and tissue distribution of cocaine and associated metabolites. Estimations of *g*-hydroxybutyrate levels in serum also use HILIC. A method for screening and confirming stimulants, narcotics, and beta-adrenergic agents in urine used the capabilities of HILIC. A comprehensive review for HILIC of seized drugs and related compounds by the Drug Enforcement Agency is also cited. The advantages of HILIC separation of isomers such as morphine-6-glucurinde and morphine-3-glucuronide are also presented. This presentation will review general theory and forensic applications of HILIC for the past 10 years.

HILIC, Forensic, HPLC

Copyright 2013 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS. ** Presenting Author*