K10 Parameter Effects in a Hydrophilic-Interaction Liquid Chromatographic Method to Simultaneously Quantify Polar Metabolites and Closely Associated Parent Opiates in Urine

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After attending this presentation, attendees will have a better understanding of the capabilities of Hydrophilic-Interaction Liquid Chromatography (HILIC). HILIC has the ability to simultaneously retain polar glucuronide metabolites while still separating structural isomers and less polar parent drugs.

This presentation will impact the forensic science community by providing information of parameter changes in a HILIC Tandem Mass Spectrometry (MS/MS) method which can simultaneously quantify 6-monoacetylmorphine (6-MAM), morphine, hydrocodone, codeine, oxycodone, hydromorphone, oxymorphone, codeine-6-glucuronide, morphine-6-glucuronide (M6G), and morphine-3-glucuronide (M3G) in urine.

HILIC has become a chromatographic tool to better retain polar analytes where reverse phase chromatography cannot. HILIC uses a mixed mode system involving a polar stationary phase and a mostly organic mobile phase with some water. The polar water component of the mobile phase favors the hydrophilic moieties of the column packing and creates a stagnant layer in which a partition occurs with the organic component of the mobile phase. This allows for polar analytes to interact with the column more and elute later.

In this presentation, zwitterion-HILIC demonstrates the ability to analyze low concentrations of polar metabolites without sacrificing selectively between the parent opiates. Zwitterion-HILIC consists of a specific stationary moiety that contains both amino and sulfate groups to allow for both positive and negative interaction with the analyte. Analytes chosen are morphine and codeine and their glucuronides, M6G, M3G and C6G. Although M3G and M6G are structural isomers, they have different pharmacological effects. When analyzed separately, M6G can provide intoxication levels and M3G can elucidate predisposition of abuse. Also hydromorphone, hydrocodone, oxymorphone, and oxycodone derivatives of morphine and codeine could be selectively separated from their associated codeine and morphine parents. 6-MAM, the primary metabolite of heroin, was also included.

HILIC parameters including buffer, pH, and concentration were evaluated on both bare silica and zwitterion-HILIC columns. Acetone and acetonitrile organic modifiers were evaluated as well. Analyte separation was achieved using the zwitterionic stationary phase with a mobile phase composed of 85% acetone and 15% ammonium formate buffer (5mM with pH of 5).

Solid-Phase Extraction (SPE) with a C18 sorbent was used to improve signal-to-noise ratio and recovery. This required a two-step elution involving a more aprotic solvent to elute the glucuronides. Electrospray Ionization (ESI) Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) was used in positive Multiple Reaction Monitoring (MRM) mode. Transitions used to evaluate M6G and M3G were 462/286 and 462/201.

It was discovered that retention and selectivity were highly influenced by how nonpolar and aprotic the organic modifier was in the mobile phase. The HILIC mechanism relies on the partition created between the stagnant water layer and the organic mobile phase. By reducing the polarity of the organic modifier, a more defined partition was created and allowed for different selectivity. The bare silica stationary phase did not provide the selectivity needed for both structural isomers and hydromorphone/hydrocodone derivatives simultaneously. Because M3G/M6G, hydrocodone/codeine, and hydromorphone/morphine pairs share the same molecular ion, retention data is needed to qualitatively identify each analyte. Retention time data Coefficient of Variations (CVs) as low as 2% demonstrated reproducibility for the qualitative determination of each analyte. Limits Of Detection (LOD) as low as 5ng/mL and Limits of Quantitation (LOQ) of 10ng/mL were observed for the morphine glucuronides. Hydromorphone and hydrocodone provided good signal-to-noise with LOD values as low as 2ng/mL and LOQ values of 10ng/mL.

The mostly organic mobile phase proved highly compatible with ionization in the ESI interface of the triple quadrupole detector. The sample preparation and compatibility to the detector allowed for a working linear range of 1ng/mL – 2,000 ng/mL with R^2 values close to 1. This method provides separation of hydrocodone and hydromorphone from parent opiates which is often overlooked when evaluating selectivity of a new morphine and codeine method.

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