Part 1: Short Review of LC Method Development Principles

Part 2: Serum Aldosterone: An LC Method Development Case Study for a Difficult Analyte

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Part 1:

This presentation will introduce the principles of reverse-phase and HILIC high performance liquid chromatography (HPLC) method development and address common problems such as column overload, injection matrix/mobile phase mismatch, and poor ionization. Best practices and maintenance suggestions will be discussed.

Part 2:

Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) has gained popularity in the clinical laboratory because of its versatility in target analytes and improvements in analytical specificity and sensitivity, and the low cost of consumable reagents on a per-test basis. However, LC-MS/MS systems do not come equipped with pre-set test menus in the manner that immunoassay instrumentation does. While there are rare exceptions, the best that a clinical laboratory can hope for in terms of pre-set methods are application notes that have not undergone clinical validation and may underperform in real-world environments. For this reason, LC-MS/MS assay development and validation is, and should be, a within-laboratory project. However, the new user can find stem to stern assay development a very daunting task.

While LC-MS/MS has much better analytical specificity than immunoassay it can suffer from the impact of interferences from isobaric compounds, gel-separator tubes, plasticizers, and unidentified compounds. Signal suppression from phospholipids also poses a challenge and may cause inaccurate results. Hence the liquid chromatography (LC) portion of the method is a critical part of developing a robust and reliable LC-MS/MS assay with adequate throughput to meet the clinical need.

This presentation will describe the LC method development for one of our more challenging to measure endogenous steroids: serum/plasma aldosterone. We will discuss the motivation for and selection of mobile phases and additives, column selection, the development of the gradient program, interference testing, and the addition of cortisol to the method. Importantly, we will cover the unexpected workflow challenges and the continual improvements and discoveries we have made along the way while the assay has been in clinical production. Other steroid methods may be described as time permits.