Introduction

Importance of pharmaceutical drug dissolution or storage form requires methods proving high accuracy and sensitivity for active and inactive compounds. FDA regulations require companies to develop methods of analysis to characterize not only the active pharmaceutical ingredient (API) but also the excipients and any degradation products that could arise from a particular synthesis process, drug substance provider, and storage conditions. UPLC methodology was selected for this study. Methods for separation and quantification of multiple analytes in an extractable mixture (e.g., related compounds, metabolites, contaminants, and impurities) are necessary for the development of drug substances. Additional steps for characterization and refinement of the drug substance are required to achieve a drug product with consistent and predictable quality. The UPLC method is the preferred method of analysis for quantifying both the API and the levels of the related compounds, contaminants, and impurities.

Experimental

Approach

In this paper, we will demonstrate the increased resolution and selectivity achievable by "Ultra Performance Liquid Chromatography" (UPLC™) and how to apply this technology for purity analysis. In particular, we will show how to manipulate selectivity and peak shape to improve resolution. By "Ultra Performance Liquid Chromatography" (UPLC™) we mean any LC system capable of being operated at reduced mobile phase flow rates that results in a unique combination of high separation efficiency and speed. UPLC is a technology designed to move ions, molecules, and particles very fast through columns to achieve high separation efficiency and speed. UPLC utilizes traditional chromatography principles, therefore method selection by applying method1 while keeping resolution constant.

Column Scouting

The different column chemistries (i.e., BEH C18, BEH Phenyl, BEH C8, BEH Shield, etc.) are well suited for purity analysis. We have performed scouting gradients of 5-90%B MeOH with 20mM Ammonium Bicarbonate pH9 buffer. Initial results show the phenyl chemistry is the most suitable for purity analysis. Impurities are labeled A - J. 5 minute scouting gradients of 5-90%B MeOH with 20mM Ammonium Bicarbonate pH9 buffer were performed. Initial results show the phenyl chemistry provides the best results. However, the BEH C18 was chosen for the final method development and future applications. The BEH C18 can be manipulated and optimized with help of the simulation software.

Manipulating Selectivity

Exploring various temperatures, hold times, and hold times of the method development, we were able to find optimum temperatures and run times that result in the desired resolution and selectivity. The following gradients and parameters were used to manipulate selectivity by traditional approach of method scouting with four UPLC Chemstation variants, various pH range, and temperature will be explored in detail. A constant resolution and detection of minor contamintion of products while keeping in minimum runtime was achieved by using the simulation software. Method optimization performed through assistance of method development simulation software (Chromatography Module, 2000).

Utilization of the development process.

Utilization of the development process results in a final method that is easier to design and troubleshoot. Further, the development process allows for easy manipulation and optimization of the method, resulting in a final method that is robust and easy to maintain.

Method Optimization Utilizing Chromatography Simulation Software

Method optimization performed through assistance of method development simulation software (Chromatography Module, 2000) would further increase the resolution.

Method Development Tools

A single quadrupole MS was configured with the FIA for peak tracking and monitoring.

In general, temperature experiments can provide peak selectivity, in some situations as selectivity is not.

DISCUSSION AND CONCLUSIONS

One goal that is often addressed in purity analysis is to optimize the UPLC method to an UPLC approach. The UPLC approach resulted in selectivity differences. Peaks C and D were chosen for UPLC degradation studies to investigate if the HPLC method could be a replacement for the UPLC method. HPLC or UPLC resulted in a ~30 minute run time vs. a 7.0 minute run time, respectively. There were other observations when performing this UPLC approach. Peaks are very narrow which may require high sampling rates. Loadability could become application specific depending on the HPLC column. Proper resolution comparisons between the two chemistries would not quite be possible.

UPLC utilizes traditional chromatography principles, therefore method selection by applying method1 while keeping resolution constant. Peak distortion due to overloading could also observed as well as the traditional PDA UV spectra. No one technique “delivers the goods”.

References