In Preparative Scale Gradient Reversed Phase Liquid Chromatography, Peak Shape and Retention as a Function of pH, Buffer Concentration, and Mass Load for Ionizable Compounds are Readily Rationalized and Addressed Based on Secondary Chemical Equilibria in the Eluent

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An ongoing challenge in preparative liquid chromatography (LC) is maintaining good chromatographic resolution and separation efficiency, particularly for basic or other ionizable compounds, during high mass loading. In an effort to address this issue, we have investigated the combined impact of different eluent pHs, buffer concentrations, and mass load on preparative scale reversed phase separations of basic compounds. It is of particular interest that improved peak shape and ability to mass load are frequently observed at pH ranges considered non-ideal (acidic and neutral) with increased buffer concentration. These surprisingly substantial performance improvements are achieved even when using buffer as many as 4 pH units on the "wrong" or ionized side of the target compound’s pK\textsubscript{a}. After thorough study, it is our assertion that these behaviors can be readily explained based on secondary chemical equilibria in solution. As pH is systematically varied over a wide range, ionizable analytes are observed to exist in 3 distinct chemical (charge) states and this is consistent with previous literature on these matters.

Use of a simple solution equilibria model readily leads to practical prediction of separation parameters that are useful and straightforward to control. This is done using peak shape as a facile way to assess and assign the adsorption type and thus charge state of the analyte in the eluent, allowing immediate assessment and adjustment of the current chromatographic conditions relative to the target analyte’s apparent pK\textsubscript{a}. In the final analysis, the ability to best load the column often seems to be a balance between adjusting pH to achieve solubility via some degree of ionization and adjusting it (and/or buffer concentration) in order to achieve a sufficiently narrow band by sending molecules through the column in a neutral or non-ionized state. An additional way to enhance mass load exploits chemical differences in apparent pK\textsubscript{a} between the chromatographic peak(s) of interest and those eluting nearby by adjustment of secondary chemical equilibria in order to achieve sufficient selectivity. Frequently, this too can be addressed effectively by controlling eluent pH and buffer concentration.

The approach of utilizing a combination of eluent pH and buffer concentration to control chromatographic behavior has been automated and this is described herein. This automated approach has been shown to be robust and successfully applied in production mode gradient reversed-phase liquid chromatography mass spectrometry (RP-LC/MS) based purification for many tens of thousands of separations, to rapidly deliver milligram to 100s of grams of material in the drug discovery setting. By broadening the range of easily accessible and programmed conditions, this automated system also facilitates the detailed study of pH and buffer effects presented here. Finally, automation of buffer choice and concentration provides a simple yet effective way to significantly save labor while simultaneously improving resolution as well as both production and chromatographic efficiency.