Waters™

5 Rules of Scaling LC Purification

Rule #2: Choose your modifier wisely

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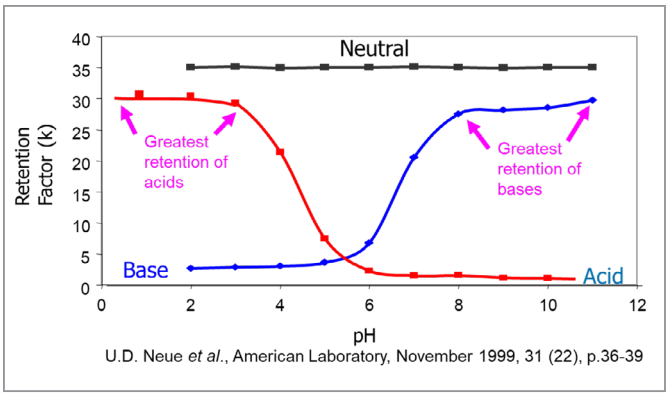
While chemists are eager to understand and develop the best practices for improving the chromatographic conditions which result in better compound resolution, peak shape, and retention at the analytical scale, these practices are even more powerful at the preparative scale when fraction triggering and compound recovery depend upon judiciously implementing them. Controlling the pH in PREP is remarkably effective for improving the quality of the separation and often leads to improved process efficiency.

Controlling pH is enormously powerful in PREP separations and should be leveraged to its maximum advantage. Un-ionized target compounds retain better on the column packing (acids at low pH, bases at high pH). Compound elution at the proper pH improves resolution, peak shape, and ultimately, product recovery. Even small changes in pH can dramatically affect chromatographic results.

Waters OBD Preparative Columns help bridge the performance gap in PREP LC with uniform, optimum packed bed density throughout the entire length and diameter of the column. Direct scalability from analytical to PREP, excellent column lifetime, and consistent column-to-column performance result in good resolution, peak shape, and mass loading for unparalleled preparative chromatography results. Trust in on-target results for successful PREP LC purification with OBD Technology.

Modifiers in reversed-phase chromatography are used to buffer or maintain the pH throughout a separation, even when gradient method conditions change. Trifluoroacetic acid, formic acid, and ammonium formate are commonly used to keep the pH acidic and below pH 5. Conversely, basic modifiers such as triethylamine (TEA), ammonium hydroxide, or ammonium bicarbonate are effective for the separation of compounds at high pH.

Modifiers can also be used to adjust the pH of the crude sample mixture to ensure that compounds are un-ionized before injection, as well as to improve the resolution of eluting peaks. Crude sample solubility may also be impacted by changes in pH. A compound is 50% ionized at its pK value (e.g., pKa or pKb). For best results, run prep separations at a pH which is about 2 pH units away from the compound's pK value. This reduces the risk of forming multiple ionized species which can complicate the separation and impact target compound recovery.



U.D. Neue *et al.*, American Laboratory, November 1999, 31 (22), p.36-39

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