

Modify selectivity in an ANP method of HPLC using different mobile phase pH - Tips & Suggestions

In Aqueous Normal Phase HPLC (ANP), perhaps the most effective and simplest way to modify selectivity is to adjust your mobile phase to a different pH.

The ionization state of the analytes and/or stationary phase is believed to play a critical part in the ANP retention mechanism, so a different pH can often produce significant changes in chromatography. In obtaining the desired pH, be sure to use a mobile phase additive that is compatible with both the stationary phase and your system; phosphates, for example, should not be used in LC-MS methods.

Common additives suitable for most applications are 0.1% formic or acetic acid and 10mM ammonium acetate or formate. The Cogent™ HPLC columns have specifications for suitable operating pH ranges. See pH specifications for the Diamond Hydride™ column and its limitations.

It has been shown that different solvents used for the organic component in ANP methods (*e.g. acetone vs. acetonitrile*) can produce notably different retention of some analytes. Data suggests that a combination of the two could be a useful method development tool in optimizing selectivity or retention. For example, a peak pair may co-elute using acetone but be well-resolved with acetonitrile; the inverse may occur for another peak pair. Using a combination of the two may be a possibility to obtain a total separation of all the peaks in this case.

