

## Buffer concentration can affect ANP retention of acids in HPLC Analyses - Tips & Suggestions

If you are using ammonium acetate or ammonium formate in an Aqueous Normal Phase (ANP) method, the concentration used may play a significant role in the retention of organic acid analytes.

Although buffer concentration may affect a chromatographic environment in numerous ways, one particularly significant effect (in the case of acids) is thought to involve adsorbed acetate or formate on a silica hydride surface. It is suggested that due to their hydrophobic nature, acetate or formate ions ***could adhere to the silica hydride surface*** and electrostatically interact with ammonia as well as analytes. In the case of acid analytes, there could be electrostatic repulsion from the acetate or formate which would reduce retention. This mechanism is still under investigation but the behavior has been observed and is a predictable model for method development of acidic analytes.

Although you should use a buffer concentration high enough to maintain adequate buffering capacity, there are numerous disadvantages to having too high a concentration for LC-MS methods. The ion source gets contaminated very quickly with higher amounts of buffer. Also peak intensity is lowered with increasing salt concentration as well.

***For these reasons, it is better to have a lower buffer concentration when possible.***

