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Under the HALO®: Importance of HPLC Mobile Phase Additives/Buffers

High-Performance Liquid Chromatography (HPLC) is one of the most widely used techniques for separating and analyzing compounds in chemical, pharmaceutical, and biological research. A key component of a successful HPLC separation is the use of mobile phase additives such as acidic modifiers, ion-pairing reagents, or even buffers in the mobile phase. Buffers play a crucial role in controlling pH, improving peak shape, influencing selectivity, maintaining compatibility with detection systems, and protecting the stationary phase. Without proper buffering, chromatographic performance can deteriorate, leading to poor resolution, inconsistent retention times, and unreliable results.

One of the most important functions of a buffer in HPLC is to maintain a constant pH. The pH of the mobile phase directly affects the ionization state of analytes, particularly those that are weak acids or bases. The ionization state determines how strongly an analyte interacts with the stationary phase and therefore influences its retention time. Even small changes in the pH can cause large variations in retention behavior. By including a buffer, the pH remains stable throughout the run, ensuring reproducible separations. Table 1 shows a list of common HPLC buffers and their respective pKa. It is important to note that buffers are most effective within ±1 pH units from their pKa values. (1) Optimal buffering concentrations are usually between 10-50mM in strength.

Buffer	pKa	UV Cutoff
Trifluoroacetic Acid*	0.3	210nm
Phosphate	2.1, 7.2, 12.3	190nm
Formic Acid*	3.8	210nm
Acetic Acid*	4.8	210nm
Carbonate*	6.4, 10.3	200nm
Ammonia*	9.2	200nm
*Volatile buffer systems, which are MS-compatible		

Table 1: Common HPLC Mobile Phase Additives

In addition to influencing chromatographic behavior, buffers must also be compatible with the detection method being used. For ultraviolet (UV) detection, buffers such as phosphate are commonly chosen because they have minimal UV absorbance. However, when using mass spectrometry (MS) detection, non-volatile buffers like phosphate can cause contamination or clogging. In these cases, volatile buffers such as ammonium acetate or ammonium formate are preferred because they evaporate easily in the MS source without leaving residues.

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Buffers also play an essential role in improving peak shape. In the absence of a stable pH, analytes may partially ionize during the separation process, causing peak tailing, broadening, or asymmetry. A well-chosen buffer minimizes these effects by keeping the analyte charge state consistent as it passes through the column. As a result, peaks become sharper and more symmetrical, which improves both qualitative and quantitative analysis. For example, figure 1 below shows a separation of beta-amyloid 1-38 injected on column with/ without the presence of ammonium formate. The addition of ammonium formate significantly improves chromatographic peak shape along with an increase in retention time. (gradient times were modified in order to match retention times)

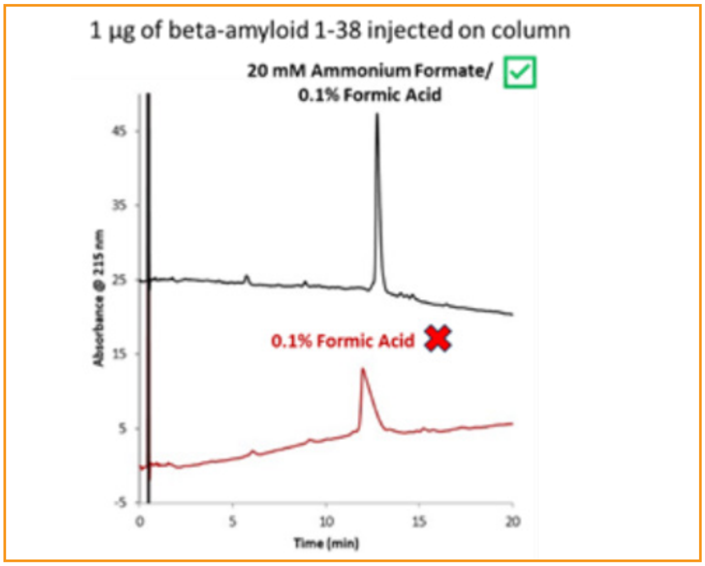

Figure 1. Analysis of beta-amyloid 1-38 injected on column with/ without the presence of ammonium formate

In summary, the use of buffers in HPLC is essential for achieving reliable, reproducible, and high-quality separations. Buffers control pH to stabilize analyte ionization, improve peak shape, influence selectivity, ensure compatibility with detectors, and protect the stationary phase. Without proper buffering, chromatographic results would be inconsistent and difficult to interpret. Therefore, selecting the appropriate buffer system is a fundamental step in developing and optimizing any HPLC method.

References:

L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography (Wiley, Hoboken, New Jersey, 3rd Ed., 2010), Chapters 2, 6 and 7.

M.W. Dong, Modern HPLC for Practicing Scientists (Wiley, Hoboken, New Jersey, 2006), Chapters 1, Chapters 1, 2, 5, 6, 8 and 10.