Reversed-phase HPLC Buffers

High-quality buffers (solutions, solids or concentrates)

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Consideration of the affects of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its affect on detection are important in reversed-phase chromatography (RPC) method development of ionic analytes. An improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reverse-phase separation of polar and ionizable compounds.

Problems, such as, partial ionization of the analyte and strong interaction between analytes and residual silanoles or other active sites on the stationary phases can be overcome by proper mobile phase buffering (maintaining the pH within a narrow range) and choosing the right ionic species and its concentration (ionic strength) in the mobile phase (1-2). In sensitive LC-MS separations that depend heavily on the correct choice of acid base buffering species and other additives (3). A buffer must be chosen based on its ability to maintain, and not suppress analyte ionization in the MS interface.

Buffer Selection

The typical pH range for reversed-phase on silica-based packing is pH 2 to 8. Choice of buffer is typically governed by the desired pH. It is important that the buffer has a pK_a close to the desired pH since buffers control pH best at their pK_a. A rule of thumb is to choose a buffer with a pK_a value <2 units of the desired mobile phase pH (see **Table 1**).

Table 1. HPLC Buffers, pK_a Values and Useful pH Range

Buffer	pK_a (25°C)	Useful pH Range
TFA	0.5	<1.5
Sulfonate	1.8	<1-2.8
Phosphate	2.1	1.1-3.1
Chloroacetate	2.9	1.9-3.9
Formate	3.8	2.8-4.8
Acetate	4.8	3.8-5.8
Sulfonate	6.9	5.9-7.9
Phosphate	7.2	6.2-8.2
Ammonia	9.2	8.2-10.2
Phosphate	12.3	11.3-13.3

Buffer Concentration: Generally, a buffer concentration of 10-50 mM is adequate for small molecules.

Buffer Solubility: A general rule is no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration.

Buffer's Effect on Detection: The choice of buffer is also dependent upon means of detection. For traditional UV detection, the buffer needs to be effectively transparent in this region, especially, critical for gradient separations. Buffers listed in **Table 1** have low enough absorption below 220 nm.

Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Phosphonate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds. With the growth in popularity of LC-MS, volatile buffer systems, such as TFA, acetate, formate, and ammonia, are frequently used due to compatibility with mass spectral (MS) detection. In regard to the issue of suppression of ionization, formate and acetate are ideal choices for positive-ion mode detection. TFA, however, can negatively impact detector response even in positive-ion mode (4,5), while it strongly suppresses ionization with negative ion mode. Acetic acid is good for negative-ion mode. LC-MS applications further limit buffer selection and buffer concentration.

References

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2. Poole, C.F. and Poole, S.K. Chromatography Today, Elsevier Science: Amsterdam, The Netherlands, 1991; 431.
3. Analytix, Five-part series on Mobile Phase Additives for LC-MS, Issue 3, 2006 (www.sigma-aldrich.com/analytix).
4. Temesi, D., Law, B., 1999, The Effect of LC Eluent Composition on MS Response Using Electrospray Ionization, LC-GC, 17:626.
5. Apffel, A. et. al. 1995. Enhanced Sensitivity for Peptide Mapping with Electrospray Liquid Chromatography-Mass Spectrometry in the Presence of Signal Suppression Due to Trifluoroacetic Acid-Containing Mobile Phases, J. Chrom. A. 712:177.

Featured Products

Description	Qty.	Cat. No.
HPLC-grade Buffers and Additives from Sigma-Aldrich/Fluka		
Ammonium acetate	50 g, 250 g	17836
Ammonium formate	50 g, 250 g	17843
Ammonium hydroxide solution in water	100 mL, 1 L	17837
Ammonium phosphate monobasic	250 g	17842
Ammonium trifluoroacetate	10 g, 50 g	17839
Potassium phosphate dibasic anhydrous	250 g	17835
Sodium formate	50 g, 250 g	17841
Sodium phosphate dibasic dehydrate	250 g	71633
Sodium phosphate monobasic anhydrous	50 g, 250 g	17844
Sodium trifluoroacetate	10 g	17840
Trifluoroacetic acid:Triethylamine 2M:1M	500 mL	09746
Trifluoroacetic acid:Triethylamine 2M:2M	100 mL	09747

For a complete list of HPLC buffers and additives, please refer to our online product catalog: ***sigma-aldrich.com***