Guideline to buffer selection for RP methods

In reversed phase liquid chromatography (RP) method development of ionisable compounds the mobile phase pH has an important influence on retention behaviour. The pH can cause drastic shifts in retention and peak symmetry if not adequately controlled. Buffers that consist of a weak acid and its conjugate base are a proper tool to keep the mobile phase pH at a nearly constant value.

The acid itself has not a sufficient buffer capacity to keep the pH constant. Most compounds contain one or more acidic or basic functional group and the stationary phase base particles themselves exhibit acidic character due to their free silanols. Therefore, for most mobile phases a controlled pH is a necessity.

Why is a stable pH value so important?

The retention of ionisable compounds on RP stationary phases strongly depends on which form is present. While the neutral form is more hydrophobic and shows high retention, the ionised form is much more hydrophilic and will therefore elute much earlier.

An acid will be >99% ionised when the pH is 2 units above its pKa and neutral at a pH at least 2 units below. Respectively, a base will be >99% ionised at a pH 2 units below its pKa and non-ionised 2 units above. So up to 2 units above or below the pKa the retention time of the analyte can drastically change even if the pH value only slightly changes.

Figure 1 illustrates the change in retention time when varying the pH for acids or bases. Acids will be more retained at low pH while bases will be more retained at high pH. Amphoteric compounds, such as peptides, are always ionised and only have a neutral net charge and therefore their highest hydrophobicity at their specific isoelectric point (pI).

In a mixture of analytes with similar structure the selectivity can change with pH and so the elution order and resolution may vary.

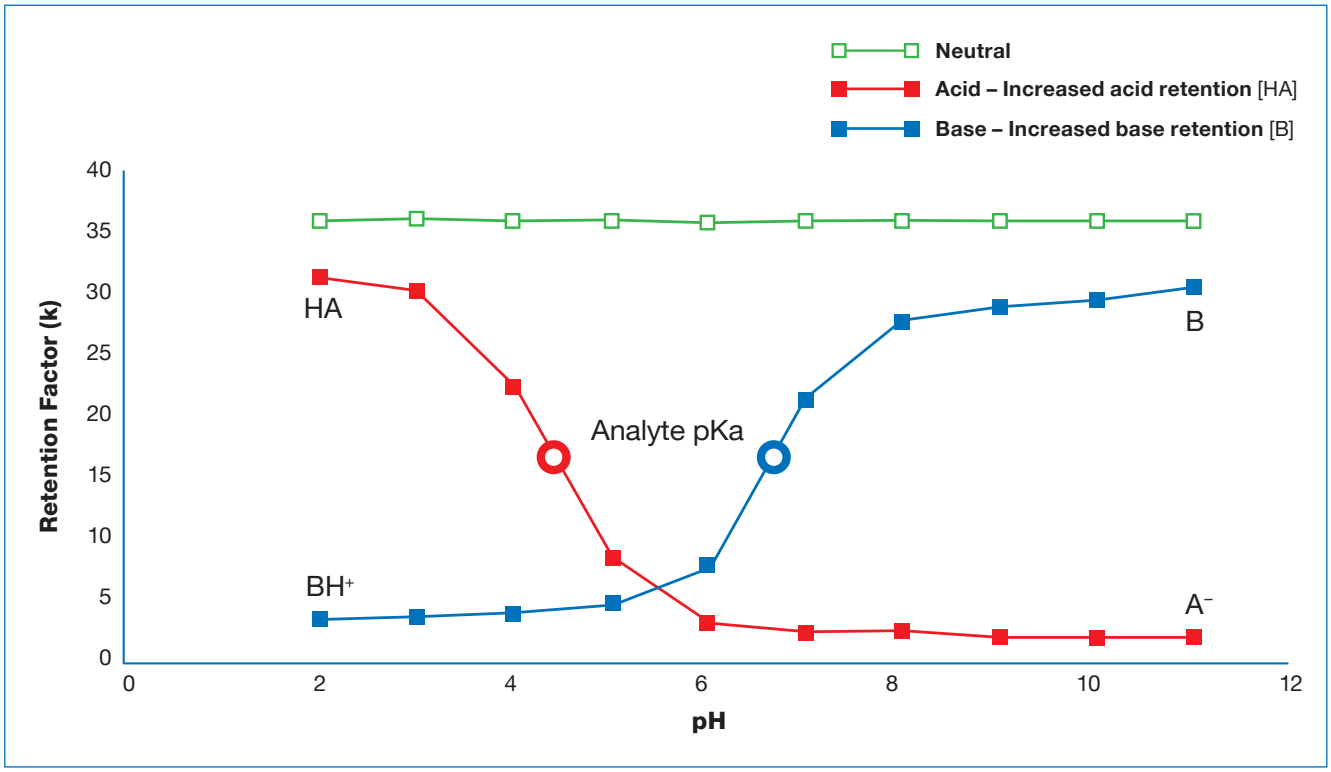

Figure 1: Illustration of the retention change when varying the pH for an acidic compound (red) and a basic compound (blue).

Buffer Selection

A buffer is most effective when it is used within ±1 pH unit of its own pKa, but can also provide proper buffering within ±2 pH units from its pKa. Table 1 shows a list of common buffers and their pKa respectively their buffering range.
Furthermore, the amount of buffer salts is crucial for the analysis and symmetric peak shape, which of course depends on the analytes and the strength of their acidic or basic functionalities. In general, just a small buffer concentration is necessary to buffer the analytes as the amount of compounds on the column is usually in the range of nanograms. In addition, the solubility of the buffer in the organic solvent must be considered. Usually, the optimal buffer concentration should be less than 25 mM. For LC/MS applications the buffer concentration should be decreased to less than 15 mM to compensate for analyte ion suppression. Furthermore, volatile buffers such as ammonium acetate have to be used in LC-MS. More MS compatible buffers are marked in Table 1.

Table 1: Buffer selection.

Buffer	pKa	Buffer range	Standard Concentration	LC/MS compatibility
TFA	<1.0		0.01~0.1%	Yes*
Phosphoric acid	2.1		0.01~0.1%	No
Ammonium dihydrogen phosphate (Na^+, K^+ salt)	2.1	1.1~3.1	5~50 mM (<20 mM recommended)	No
Sodium dihydrogencitrate	3.1	2.14.1	<25 mM	No
Formic acid	3.7		0.1~1.0%	Yes
Ammonium formate (Na^+, K^+ salt)	3.7	2.7~4.7	5~50 mM	NH_4^+ salt: Yes [Na^+, K^+ salt: No]
Sodium hydrogencitrate	4.7	3.7~5.7	<25 mM	No
Acetic acid	4.8		0.5~5.0%	Yes
Ammonium acetate (Na^+, K^+ salt)	4.8	3.8~5.8	5~50 mM	NH_4^+ salt: Yes [Na^+, K^+ salt: No]
Sodium citrate	5.4	4.4~6.4	<25 mM	No
Ammonium hydrogen phosphate (Na^+, K^+ salt)	7.2	6.2~8.2	5~50 mM (<20 mM recommended)	No
Triethanolamine	7.8	6.8~8.8	<15 mM	Yes
TrisBase	8.3	7.3~9.3	<15 mM	Yes
4-Methylmorpholine	8.4	7.4~9.4	< 10 mM	Yes
TEAA		4.6~6, 10~11	<20 mM	Yes
Ammonium formate, ammonium acetate	9.2	8.2~10.2	<20 mM	Yes
Sodium borate	9.2	8.2~10.2	<25 mM	No
Ammonium bicarbonate		8.5~10.5	<10 mM	Yes
CAPSO	9.7	8.7~10.7	<25 mM	No
Glycine	2.4, 9.8	8.8~10.8	<25 mM	No
1-Methylpyridine	10.2	9.2~11.2	1~10 mM	Yes
CAPS	10.4	9.4~11.4	<25 mM	No
Diethylamine	10.5	9.5~11.5	<15 mM	Yes
n-Hexylamine	10.6	9.6~11.6	<15 mM	Yes
n-Butylamine	10.8	9.8~11.8	<15 mM	Yes
Dibutylamine	11.3	10.3~12.3	<15 mM	Yes
Pyrrolidine	11.3	10.3~12.3	<15 mM	Yes
Sodium phosphate, potassium phosphate	12.3	11.3~13.3	<10 mM	No

**Limited LC/MS compatibility due to ion suppression*

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