Chromatography Solutions

Chromatography white paper

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Considerations and best practices for mobile phase buffer selection and pH control for LC and LC-MS method development

When developing a new LC or LC-MS separation, careful consideration of mobile phase composition is essential for optimising peak shape, improving separation selectivity, and ensuring method robustness. For samples containing ionisable compounds, it is important to select an appropriate mobile phase pH to control the analyte ionisation state, in order to achieve reliable retention times. Buffers are commonly used in reversed-phase separations to tightly control the mobile phase pH, to improve method robustness, and to suppress undesirable analyte interactions with the silica surface.

For LC methods, buffer selection requires consideration of both the analyte and buffer salt properties. Incorrect buffer selection can result in problematic methods exhibiting poor peak shape, retention time shifts and reproducibility issues. Additionally, the analyst must ensure that the buffer is compatible with the detection mode and analytical column used for the separation. This white paper discusses the important aspects to consider when selecting mobile phase buffers to ensure the development of robust and reproducible LC separations.

Avantor® ACE®

WHY IS MOBILE PHASE PH IMPORTANT?

In reversed-phase separations, analyte retention is largely determined by analyte hydrophobicity. For ionisable analytes, as the degree of ionisation increases, retention typically decreases (providing that no alternative modes of interaction such as ion exchange are present). The degree of analyte ionisation is determined by the mobile phase pH and analyte pK_a. This means that mobile phase pH can have a profound effect on how strongly some analytes are retained, and on the separation ultimately obtained. Selecting a suitable mobile phase pH is, therefore, vitally important when developing methods for samples containing ionisable analytes.

In many cases, small changes in the mobile phase pH can have a significant effect on a separation, as shown in Figure 1. In this example, a small change in the mobile phase pH of just 0.1 pH units results in complete loss of resolution between peaks 3 and 4. Clearly, this separation shows a lack of robustness and could prove to be problematic during the method lifetime due to inter- and intra-laboratory variations in mobile phase preparation. In cases like this, it is important to carefully consider and optimise the mobile phase pH during method development to, not only ensure full analyte separation, but also to provide a robust and usable method.

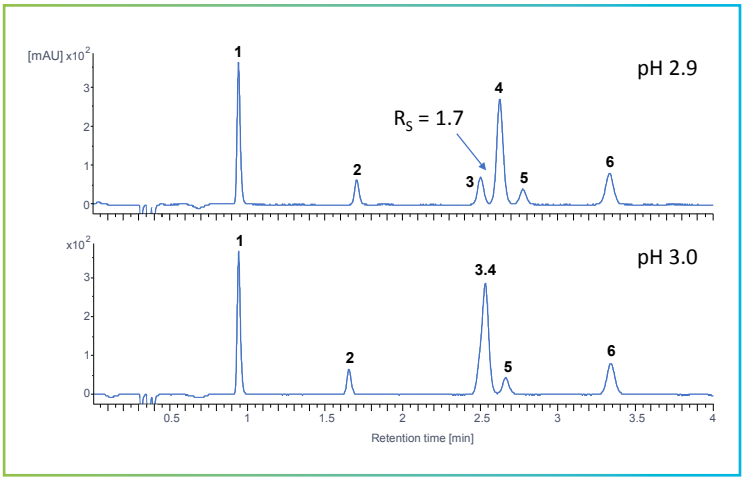

FIGURE 1:
Chromatograms showing the effect of a small change in pH on a benzoic acid derivative separation.
Column: Avantor® ACE® UltraCore 2.5 SuperC18, 100x3,0 mm; Mobile phase: 20 mM ammonium formate in MeCN/H_2O 15:85 v/v; Flow rate: 1,20 ml/min; Injection volume: 5 µl; Temperature: 40 °C; Detection: UV, 214 nm. Sample: 1. 3-Hydroxybenzoic acid, 2. 4-Cyanobenzoic acid, 3. Benzoic acid, 4. 3-Nitrobenzoic acid, 5. 4-Nitrobenzoic acid, 6. 4-Methoxybenzoic acid.

For methods that are highly sensitive to small changes in pH, the use of a correctly buffered mobile phase will help to minimise the potential impact of small variations in pH.

Another important consideration is the effect of mobile phase pH on unbonded acidic silanol groups at the silica surface. On older 'type-A' silicas, these silanol groups have pK_a values in the region of pH 4 to 5[1]. At mobile phase pH's above pH 6.0, significant silanol ionisation can occur. Interaction of these negatively charged silanol groups, with positively charged basic analytes, is a major historical cause of peak tailing for bases in reversed-phase LC. Higher purity 'type-B' stationary phases typically have less acidic silanol activity (pK_a approx. pH 7), leading to significantly improved peak shape and improved reproducibility. This is highlighted in Figure 2, which compares the peak shape for basic analytes on the Avantor® ACE® C18 and a column packed with 'type-A' silica. The Avantor® ACE® C18 shows vastly improved peak shape due to the highly inert, ultra-pure silica used in the column manufacturing process. It is highly recommended that any new method is developed using a modern 'type-B' column, such as the Avantor® ACE® reversed-phase HPLC and UHPLC columns.

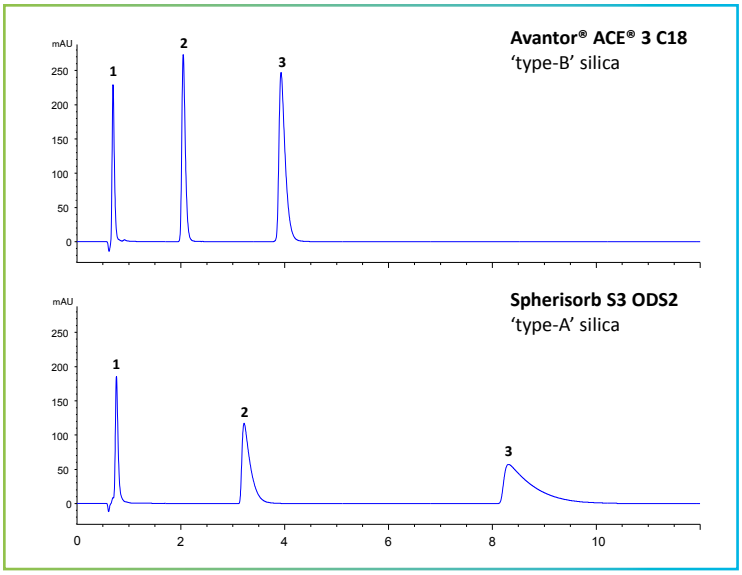

FIGURE 2:
Comparison of peak shape for basic analytes chromatographed at pH 7,6 on a modern, high purity 'type-B' silica column (Top) and an older, low purity silica (Bottom).
Column dimensions: 50x2,1 mm; Mobile phase: 20 mM KH_2PO_4 pH 7,6 in MeOH/H_2O 75:25 v/v; Flow rate: 0,2 ml/min; Injection volume: 2 µl; Temperature: 30 °C; Detection: UV, 214 nm; Sample: 1. Salbutamol, 2. Diphenhydramine, 3. Imipramine.

UNDERSTANDING HOW MOBILE PHASE PH AFFECTS ANALYTE RETENTION

To understand how the mobile phase pH may affect analyte retention, it is good practice to assess known analyte properties prior to beginning method development. The analyte pK_a allows the chromatographer to assess how the ionisation state of an analyte varies as a function of pH. This information can be used to define the most appropriate mobile phase pH for the separation. Figure 3 demonstrates how the ionisation state of simple acidic and basic analytes varies according to pH. For basic analytes, at mobile phase pH's below their pK_a, the analyte will be predominantly positively charged. At high pH (above their pK_a), they will be in their neutral form, and will be better retained by reversed-phase. Conversely, acidic species show their strongest retention with a mobile phase below their pK_a and are more weakly retained at high pH, in their deprotonated form.

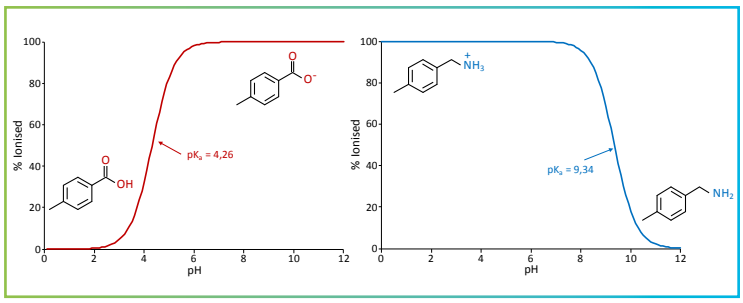

FIGURE 3:
Plot of percentage ionisation vs pH for a simple acidic analyte (red) and a simple basic analyte (blue).

When the mobile phase pH equals the analyte pK_a, the analyte is considered to be 50% ionised and 50% unionised. From here, a small change in pH (for example 0.5 pH units) will result in a comparatively large change in the analyte's ionisation state and, therefore, retention time. This may result in a non-robust method; any small change in pH, e.g. variations in mobile phase preparation, will result in a shift in the analyte's retention. A more robust method would be achieved at a pH well away from the analyte pK_a. As a general rule, it is recommended that, if possible, the mobile phase should have a pH of ±2 pH units from the critical analyte's pK_a.

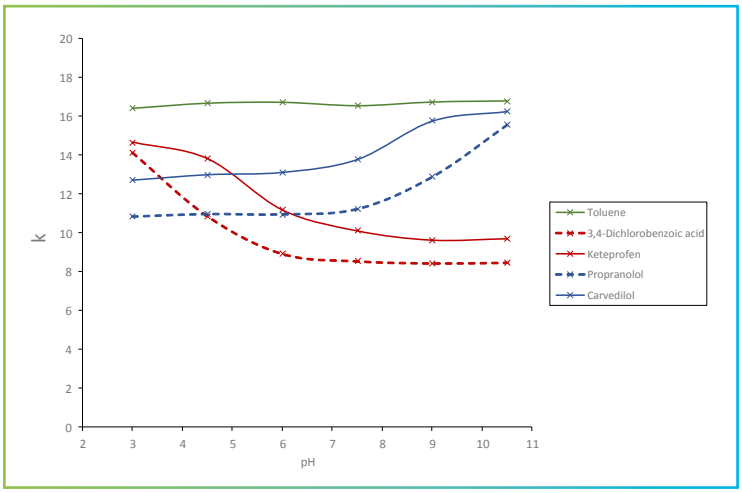

FIGURE 4:
Effect of mobile phase pH on analyte retention factor (k) on a high pH stable Avantor® ACE® SuperC18 column.
Column: Avantor® ACE® Excel 3 SuperC18, 50x2,1 mm; Mobile phase: A: 20 mM ammonium formate pH 3,0; 4,5; 6,0; 7,5; 9,0 and 10,5 (aq), B: 20 mM ammonium formate pH 3,0; 4,5; 6,0; 7,5; 9,0 and 10,5 in MeCN/H_2O 9:1 v/v; Gradient: 3 to 100% B in 5 minutes; Flow rate: 0,6 ml/min; Injection volume: 1 µl; Temperature: 40 °C; Detection: UV, 214 nm.

Figure 4 demonstrates experimentally how the mobile phase pH affects the retention of a set of acidic, basic and neutral analytes. Toluene does not contain any ionisable functionality and is neutral across the entire pH range. Therefore, mobile phase pH has no significant effect on its retention. At low pH, acidic analytes (3.4-dichlorobenzoic acid and ketoprofen) are in their neutral, non-ionised form and, therefore, show their strongest retention. As the pH is increased, the degree of ionisation increases, and retention gradually decreases. In contrast, basic analytes (propranolol and carvedilol) are positively charged at low pH, and consequently show shorter retention. As the pH increases, the ionisation is suppressed, and analyte retention increases.

From Figure 4, it is clear when ionisable analytes are present in a sample, the selectivity between analytes can vary significantly with pH. For such samples, it is highly recommended that mobile phase pH is explored during initial method development, to determine the most suitable value. When beginning any method development, it is useful to consider analyte structures and properties, to anticipate any acidic/basic functionality with the ability to ionise. If these properties are unknown, screening the sample on a generic gradient using several mobile phase pH values can be a productive starting point.

HOW TO CONTROL MOBILE PHASE PH AND FACTORS AFFECTING ADDITIVE / BUFFER CHOICE

A variety of mobile phase additives can be used to control the mobile phase pH. Table 1 lists some of the most commonly used additives. In many cases, where for example an acidic mobile phase is required at a pH well away from the analyte pK_a, a simple mobile phase containing a low percentage by volume of the additive may be sufficient. For example, mobile phases containing 0,1% v/v formic acid or phosphoric acid are commonly used. Similarly, for high pH work 0,1% v/v ammonium hydroxide is useful. For peptide and protein separations, 0,1% v/v TFA is a commonly used ion-pairing additive. For simple mobile phases containing only additives, it is important to use ultra-high purity LC columns, such as Avantor® ACE®, for optimum results.

Table 1: Common mobile phase additives.[2,3,4]

	pK_a (25 °C)	LC-MS compatible	UV cut-off (nm)
Trifluoroacetic acid (TFA)	0,3	●	210 (0,1%)
Phosphoric acid	2,15; 7,20; 12,33		<200 (10 mM)
Citric acid	3,13; 4,76; 6,40		230 (10 mM)
Formic acid	3,75	●	210 (10 mM)
Acetic acid	4,76	●	210 (10 mM)
Ammonium hydroxide	9,3	●	<200 (10 mM)
Triethylamine	10,8	●	<200 (10 mM)

Often, separations involving ionisable analytes can be highly sensitive to small variations in pH, and the use of a buffered mobile phase is required. A correctly buffered mobile phase will resist any small changes in pH, (e.g. through absorption of CO_2, or from errors in the mobile phase preparation). A buffered mobile phase consists of a dissolved buffer salt that is pH adjusted using an appropriate acid or base. Table 2 shows some of the most commonly used reversed-phase buffer systems. When used within the stated pH range, 10 to 50 mM concentrations have a high buffering capacity and can be used for highly robust control of the pH.

For LC analyses with UV detection, phosphoric acid combined with its sodium or potassium salts are a common choice, as the multiple suppress cover a large pH range (although practical limits exclude the upper pK_a). The gap in buffering capacity between pH 3.1 to 6.2 is conveniently filled by ammonium acetate / acetic acid. Citrate also possesses three overlapping pK_as however, its use is somewhat limited by its comparatively high UV cut-off.

Table 2: Common reversed-phase mobile phase buffers.[2,3,4]

	pH range	LC-MS compatible	UV cut-off (nm)
Phosphate / phosphoric acid (pK_{a1})	1,1 – 3,1		<200 (10 mM)
Citrate / citric acid (pK_{a1})	2,1 - 4,1		230 (10 mM)
Ammonium formate / formic acid	2,7 - 4,7	●	210 (10 mM)
Citrate / citric acid (pK_{a2})	3,7 - 5,7		230 (10 mM)
Ammonium acetate / acetic acid	3,8 – 5,8	●	210 (10 mM)
Citrate / citric acid (pK_{a3})	4,4 – 6,4		230 (10 mM)
Ammonium bicarbonate (pK_{a1})	5,4 - 7,4	●	<200 (10 mM)
Phosphate / phosphoric acid (pK_{a2})	6,2 - 8,2		<200 (10 mM)
Ammonium bicarbonate (pK_{a2})	9,3 - 11,3	●	<200 (10 mM)
Phosphate / phosphoric acid (pK_{a3})	11,3 – 13,3		<200 (10 mM)

For analyses using detectors such as Mass Spectrometry (MS) and Evaporative Light Scattering Detectors (ELSD), it is essential to use volatile buffers; non-volatile buffers such as phosphate will precipitate within the detector. In these cases, volatile additives such as formic acid, ammonium hydroxide, formate, acetate and ammonium bicarbonate buffers are commonly used.

MOBILE PHASE AND BUFFER PREPARATION

A typical buffer can potentially be prepared using several different approaches, which are summarised in Table 3.
To obtain reproducible separations and minimise the potential for inter-lab variation, the mobile phase and buffer preparation should be accurately recorded in documented procedures.

Perhaps the most reproducible method to prepare buffers is to weigh the individual buffer components according to a prescribed recipe, and then dilute them to the required volume with water.[1,5] This gravimetric approach ensures that the resulting buffer has a fixed concentration and pH, although it is worthwhile checking the pH of the final buffer solution for accuracy. Alternatively, the buffer could be prepared by mixing separate equimolar solutions of the acid and buffer salt (e.g. 15 mM formic acid and 15 mM ammonium formate). The pH is monitored as the two solutions are mixed until

Table 3: Summary of some commonly used approaches to preparing mobile phase buffers.

	Approach			
	1. Gravimetric	**2. Blending equimolar solutions**	**3. Titration with concentrated acid / base**	**4. Stock buffer**
Procedure	Weigh prescribed amount of buffer salt and acid / base. Dilute to specified volume with water.	Prepare separate solutions of the acidic and basic buffer components (e.g. formic acid and formate salt). Blend the solutions to achieve desired pH.	Weigh and dissolve the specified amount of buffer salt in ~900 ml of water. Adjust the pH to specified pH using concentrated acid / base. Make up to 1000 ml with water.	Prepare an aqueous stock buffer (e.g. 200 mM) at the specified pH using approach 1, 2 or 3. Dilute an aliquot of the stock solution with prescribed amount of water and organic solvent.
Benefits	Most accurate and reproducible preparation method. Final buffer has a fixed concentration and pH. The final pH should be measured for confirmation.	Provides buffer with correct concentration and accurate pH.	Straightforward and reliable. Buffer concentration is higher than equimolar approach. Accurate target pH.	Concentrated stock buffers tend to have longer shelf life. Fewer time-consuming buffer preparations. Can be used to prepare multiple mobile phases with different concentrations of organic solvent.

the desired pH is reached. The resulting buffer solution will have the specified pH and concentration. One of the most widely used approaches is to dissolve the buffer salt in water (approximately 90% of the final volume required), and adjust the pH using concentrated acid or base to the desired pH value before making up to the final volume with water.[5] The final buffer will have a higher concentration than the equimolar approach, but will have the correct pH.

As a practical note, it is important to always make any pH measurement or adjustments at the appropriate stage of mobile phase preparation. The pH of the buffer / mobile phase should never be adjusted or measured after the organic modifier is added. Although a pH measurement can be made, it will not be numerically comparable to aqueous pH values.

A useful approach for preparing buffers, especially if a buffer is used frequently, is to prepare a concentrated stock buffer solution that can be diluted to formulate the required mobile phase. This approach has several advantages; the number of lengthy buffer preparations can be minimised and additionally, concentrated buffer solutions can typically be stored for longer periods of time under suitable conditions. Finally, the stock solution can be used to prepare mobile phases containing different organic modifier concentrations. For example, a mobile phase containing 10 mM ammonium formate pH 3.0 in 1 litre of MeOH:H_2O 50:50 v/v can be prepared by combining 50 ml of 200 mM ammonium formate pH 3.0 with 450 ml water and 500 ml of methanol. The same volume of stock buffer could also be combined with, for example, 800 ml of water and 150 ml of methanol to produce a mobile phase of 10 mM ammonium formate in MeOH:H_2O 15:85 v/v. When using this approach, it is advisable to combine the aqueous portions of the mobile phase first, followed by addition of the organic modifier. Addition of concentrated buffer directly into the organic solvent can lead to buffer precipitation.

Precipitation of the buffer when preparing the mobile phase, or within the LC system, can cause blockages in the column or system, or potentially damage LC system components. It is, therefore, important to consider the solubility of buffer salts in the mobile phase as some buffers have greater solubility than others (for further details regarding buffer solubility in organic solvent, please refer to Table 4 in reference 1). For example, potassium dihydrogen phosphate shows substantially reduced solubility in over 60% organic, whereas ammonium formate is readily soluble in 90% organic. The solubility of a buffer salt is dependent on the counterion, with inorganic counterions generally being less soluble than organic equivalents. In addition, buffers tend to be more soluble in methanol than acetonitrile. For isocratic methods using a high percentage of organic in the mobile phase, or gradient methods that finish at a high organic percentage, care should be taken.

These considerations are also important when separate preparations of the buffer and individual mobile phase components are mixed online. For example, 10 mM potassium phosphate in MeCN:water 50:50 v/v could be mixed online using 20 mM potassium phosphate on line A and 100% MeCN on line B. In this case, although 10 mM potassium phosphate is soluble in the final composition after mixing, buffer precipitation could occur at the point where the concentrated buffer meets the pure organic solvent. An alternative approach would be to use 10 mM potassium phosphate on line A and the same concentration of buffer dissolved in

MeCN:water 60:40 v/v on line B. The two can then be blended to produce the desired mobile phase concentration.

In HILIC mode, the use of high concentrations of acetonitrile in the mobile phase (typically 70 to 90%) restricts the use of many buffer salts. Only buffers that are highly soluble in organic solvent are suitable for use. Ammonium formate and ammonium acetate both have good solubility in organic solvent and can be used successfully in HILIC separations.[6] The use of inorganic buffers, such as phosphate, is not recommended for HILIC separations.

BUFFER CONCENTRATION

Buffer concentrations in the range of 5 to 50 mM are common. In general, a buffer concentration of at least 5 to 10 mM is recommended to ensure sufficient buffering capacity to provide robust pH control. In addition to controlling pH, the buffer can also help to pK_as ionic interactions between charged analytes and silanol groups on the silica surface and improve peak shape.[7] Modern columns manufactured from type-B silica contain fewer acidic silanol groups than older generation columns, and can typically be used successfully with lower buffer concentrations. Often, the ability to use lower additive concentrations is highly beneficial. For example, when analysing proteins and peptides by LC-MS, TFA is often utilised as an additive in the mobile phase to improve analyte peak shape and retention. However, TFA can suppress the MS signal and has a detrimental effect on sensitivity. Figure 5 shows how, by using an Avantor® ACE® column which is manufactured using ultra-inert base silica, the concentration of TFA can be drastically reduced whilst still maintaining excellent peak shape. In contrast, when an older, lower purity column is used, peptide peak shape rapidly deteriorates as the TFA concentration is reduced. This is another advantage provided by modern, ultra-high purity columns, such as Avantor® ACE® columns.

Although it may be tempting to minimise the buffer concentration wherever possible, it is worth considering that buffer concentration can be a useful parameter for fine tuning the selectivity of a separation during method development. Figure 6 shows the effect of buffer concentration on the separation of catecholamines on an Avantor® ACE® C18-PFP column using a 100% aqueous mobile

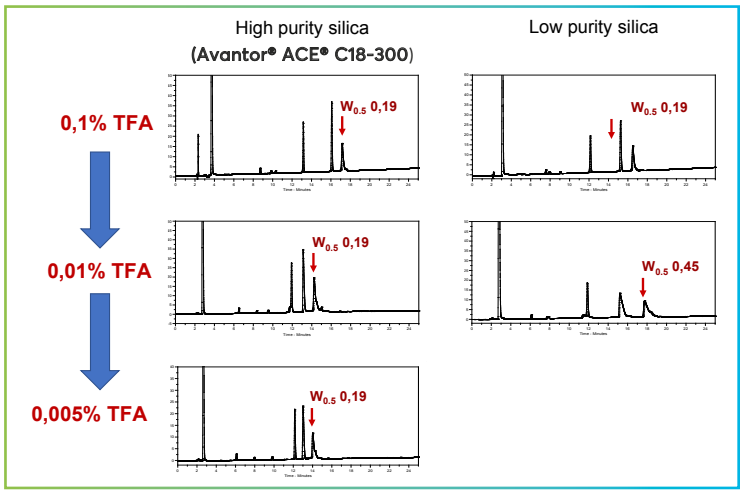

FIGURE 5: **Effect of TFA concentration on the peak shape of a peptide separation performed on a column manufactured using modern, high purity silica (Avantor® ACE® C18-300) and a low purity silica column.**
Column dimensions: 250x4,6 mm, 5 µm, C18 300 Å; Mobile phase: A: TFA (aq), B: TFA in MeCN (% TFA as specified above); Gradient: 10 to 55% B in 37,5 minutes; Flow rate: 1,5 ml/min; Detection: UV, 200 nm; Sample: 1. Gly-Tyr, 2. Oxytocin, 3. Angiotensin II, 4. Neurotensin.

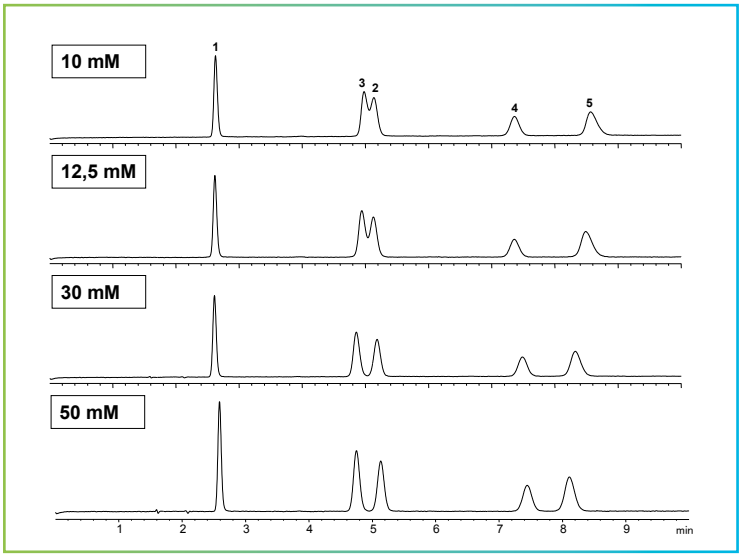

FIGURE 6: **Effect of buffer concentration on the separation of catecholamines on an Avantor® ACE® 5 C18-PFP column.**
Column dimensions: 150x4,6 mm; Mobile phase: Ammonium formate pH 3,0 (aq); Flow rate: 1,0 ml/min; Injection volume: 5 µl; Temperature: 22 °C; Detection: UV, 266 nm; Sample: 1. Norepinephrine, 2. Levodopa, 3. Epinephrine, 4. Tyrosine, 5. Dopamine.

phase. In this example, at low buffer concentrations, insufficient separation is obtained between levodopa and epinephrine. As the buffer concentration is increased, the separation is improved. This example demonstrates that, although not typically used as a primary method development parameter, assessing buffer concentration can be useful.

COLUMN CONSIDERATIONS

As discussed, it is highly recommended that columns manufactured from modern, high purity silica are used for the development of new methods to improve peak shape and reproducibility. Additionally, fresh columns should be used when beginning method development, to ensure that previous methods run on the column have not altered the characteristics of the stationary phase. This is especially important when ion-pairing additives, such as TFA have been used, as these can alter the selectivity of the column and may be impossible to fully remove. Not following this approach may lead to methods that were successfully developed on an old column but go on to fail when run on a fresh column.

When selecting a mobile phase pH and buffer for a new method, it is important to consider column stability. Generally, most silica columns should be used between a pH of 2 and 8 for optimum column lifetime. At low pH, hydrolysis can lead to stationary phase loss, whereas beyond pH 8, silica dissolution can occur. Both processes are accelerated at higher temperatures. Consequently, many reversed-phase columns cannot be used with high pH mobile phases. A number of columns that can tolerate high pH are commercially available and are typically manufactured from a hybrid organo-silica material, polymer-based, or utilise modified bonding technology. The Avantor® ACE® Excel SuperC18, Avantor® ACE® UltraCore SuperC18 and Avantor® ACE® UltraCore SuperPhenylHexyl phases are novel stationary phases that have been developed for use over a wider pH range. The use of proprietary Encapsulated Bonding Technology (EBT™) during the manufacturing process means that these phases can be used with mobile phases over an extended pH range of pH 1,5 to 11,0 (pH 1,5 to 11,5 for the Avantor® ACE® Excel SuperC18). The ability to work with a wider range of mobile phase pH's provides the chromatographer with increased flexibility to fully explore pH as a method development tool when working with ionisable analytes.[8] Figure 7 demonstrates how the ability to investigate high pH mobile phases during method development can be a powerful approach when working with ionisable compounds. In this case, a high pH mobile phase provides, not only significantly better selectivity and analyte resolution, but also improved retention and peak shape for the basic analytes.

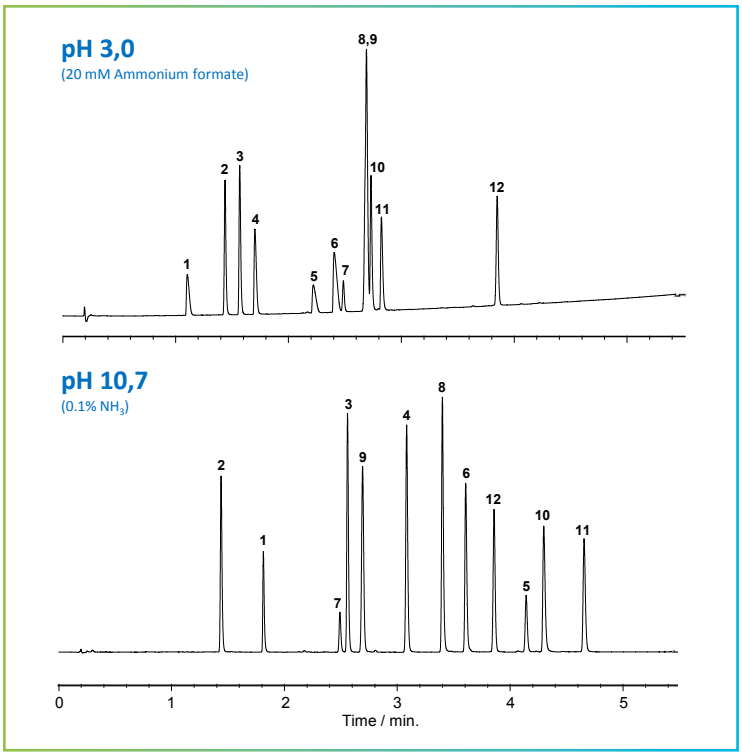

FIGURE 7: **Separation of a sample containing neutral and ionisable analytes using low and high pH mobile phases on an Avantor® ACE® UltraCore 2.5 SuperC18 column.**
Column: Avantor® ACE® UltraCore 2.5 SuperC18, 50x2,1 mm; Mobile phase (low pH): A: 20 mM ammonium formate pH 3,0 (aq), B: 20 mM ammonium formate pH 3,0 in MeCN/H_2O 9:1 v/v; Mobile phase (high pH): A: 0,1% NH_3 (aq) B: 0,1% NH_3 in MeCN/H_2O 9:1 v/v; Gradient: 3 to 100% B in 5 minutes; Flow rate: 0,60 ml/min; Injection volume: 1,5 µl; Temperature: 40 °C; Detection: UV, 254 nm.

ADDITIONAL TIPS AND GOOD PRACTICES

It is important to always use high purity buffer salts, additives and solvents (HPLC grade or better) when making up LC mobile phases. Buffer purity tends to be more critical for gradient separations than isocratic separations, as any buffer impurities can build

up on the column and are then eluted as impurity peaks as the gradient progresses. Buffer solutions and mobile phases can also be filtered to remove any dust or particulates.

For gradient methods using mobile phases containing buffers or additives, it is highly recommended that a buffer or additive is included in equal concentration in both mobile phases. Often, the buffer or additive is included in only the A line, with organic solvent on the B line. This approach creates a buffer concentration and / or pH gradient throughout the gradient program, which can cause issues with method reproducibility and robustness. In addition, for methods using UV detection, if the additive absorbs significantly at the detection wavelength, then a sloping baseline can result, as shown in Figure 8. This situation is readily resolved by incorporating the additive into the B line.

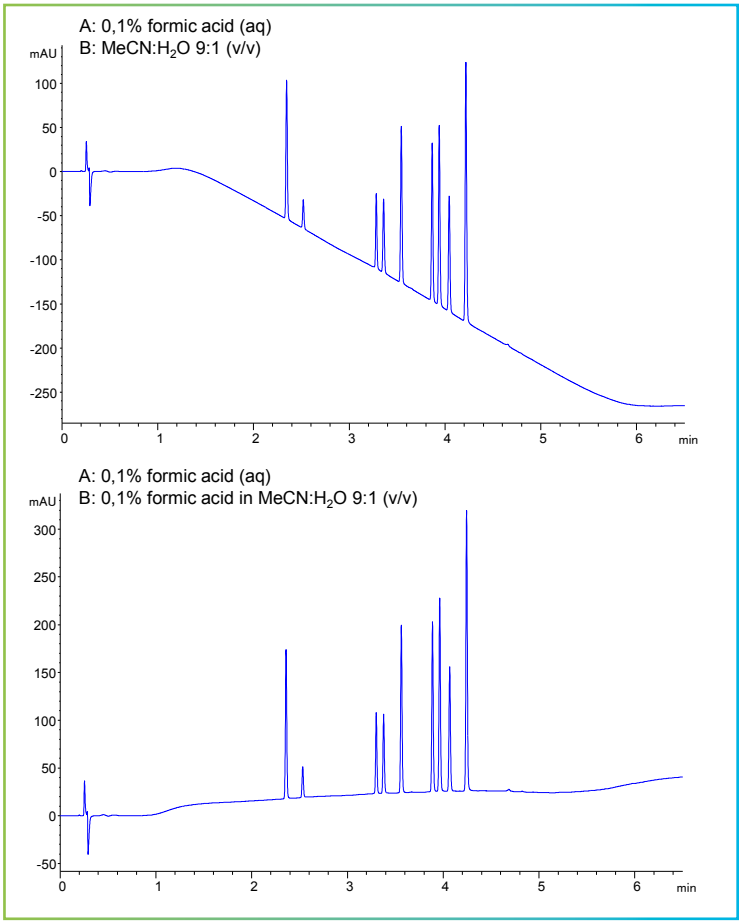

FIGURE 8: Gradient separation of non-steroidal anti-inflammatory drugs on an Avantor® ACE® Excel C18-PFP column, showing the effect of including the mobile phase additive in the A line only (top) and both the A and B lines (bottom).
Column: Avantor® ACE® Excel 2 C18-PFP, 50x3,0 mm; Gradient: 5 to 100% B in 5 minutes; Flow rate: 1,20 ml/min; Injection volume: 1 µl; Temperature: 40 °C; Detection: UV, 214 nm. Sample (in order of elution): 1. 2-Acetoxybenzoic acid, 2. Phenacetin, 3. Sulindac, 4. Tolmetin, 5. Naproxen, 6. Flurbiprofen, 7. Diclofenac, 8. Phenylbutazone, 9. Meclofenamic acid.

Microbe growth is a potential issue for aqueous solutions of buffers. It is always important to set appropriate expiration dates for buffer preparations. In general, lower concentration buffers at neutral pH's will have shorter expiration times than concentrated stock solutions of acidified buffers.

As a final note, it is always good practice to remove buffers from the LC system after use to help reduce the risk of buffer precipitation and microbial growth. This can be accomplished using a 50:50 water:organic mobile phase. Likewise, it is important to remove additives and buffers from the LC column before storage to help prolong column lifetime. For reversed-phase columns, this should not be performed with 100% water, as this could lead to de-wetting, or phase collapse, of the stationary phase.

CONCLUSIONS

When developing new LC and LC-MS separations, it is important to carefully consider the mobile phase composition to control and optimise analyte retention, selectivity and peak shape. For ionisable analytes, mobile phase pH is a critical parameter which can dramatically affect retention. Mobile phase buffers are commonly used to control mobile phase pH and help to achieve robust and reproducible separations. The wide range of buffers and additives that are available can make choosing the right mobile phase a complicated and daunting task. However, careful consideration of a few key concepts can help to ensure that the appropriate buffer is selected and can dramatically influence the success of method development.

This white paper has outlined how consideration of analyte properties can help the analyst to better understand how retention is

affected by pH, and how to select the most suitable buffer. Important practical considerations, including buffer solubility, concentration, usable pH range and compatibility with different detection modes, have been additionally discussed. Importantly, several different approaches to mobile phase preparation are often encountered. This means that it is important to consider and accurately document procedures to ensure that methods can be reliably reproduced and operated by other laboratories and users. Finally, the use of modern, ultra-pure LC stationary phases, such as Avantor® ACE®, can help to improve method reproducibility and robustness, thereby minimising the risk of method failure throughout the method lifecycle.

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