Issues with basic analytes

Analysis of basic compounds using reversed phase columns can be quite challenging. Quite often peak tailing is observed, as secondary interactions between the stationary phase and the analyte occur. Fortunately, there are several techniques to prevent it successfully.

1. pH adjustments

The most common way to reduce peak tailing of basic compounds is the adjustment of the pH of the mobile phase. The solution pH and the analyte pKa both determine the ionisation status of the analyte. If pH = pKa the compound is present in ionised and non-ionised forms in equal parts. If the pH is higher than the pKa of the basic compound a higher ratio is in its non-ionised form, up to 100% if the pH is high enough. Conversely, the amount of ionised compound will be greater if the pH is below the compound pKa. For the retention on a reversed phase column, it is recommended to work two pH units above the analyte pKa to ensure that the compound is present in a neutral state, which means that it is more hydrophobic and therefore has increased retention.

2. Buffer

However, sometimes it is not possible to work at alkaline pH or the analyte pKa is too high to follow the 2 pH unit rule. In this case, the basic compound will at least partially be present in its protonated form, which means that secondary interactions with the opposite charged surface of the stationary phase can take place. To overcome this issue different approaches can be made. On the one hand, the solution pH can be decreased to pH=2–3 which leads to residual silanols being protonated and therefore non-charged under these conditions. On the other hand, it can be useful to use a buffer in the mobile phases. Buffers not only control the solution pH, but the buffer salt ions also interact with the free residual silanols which compete with and thereby reduce the secondary interaction with the analyte. Increasing the ionic strength can be an effective tool to mask these secondary interactions, but please be aware of the solubility of the analyte at higher ionic strength.

3. Ion-pairing agents

Another possible method to reduce peak tailing of basic compounds is the use of ion-pairing agents. Ion-pairing agents contain both an ionic functional group as well as a hydrophobic group. The ionic part “pairs” with the ionised base, forming a pseudo-neutral complex with the analyte and the hydrophobic part provides stronger retention to the stationary phase. Ion-pairing agents for basic compounds need to be negatively charged and so commonly used agents are acids such as hexane-, heptane- or octane sulfonic acid or trifluoroacetic acid.

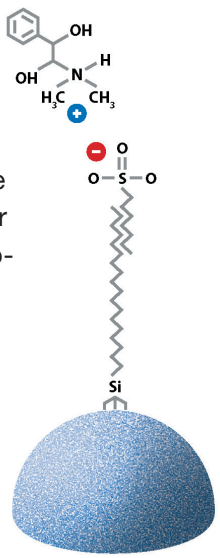
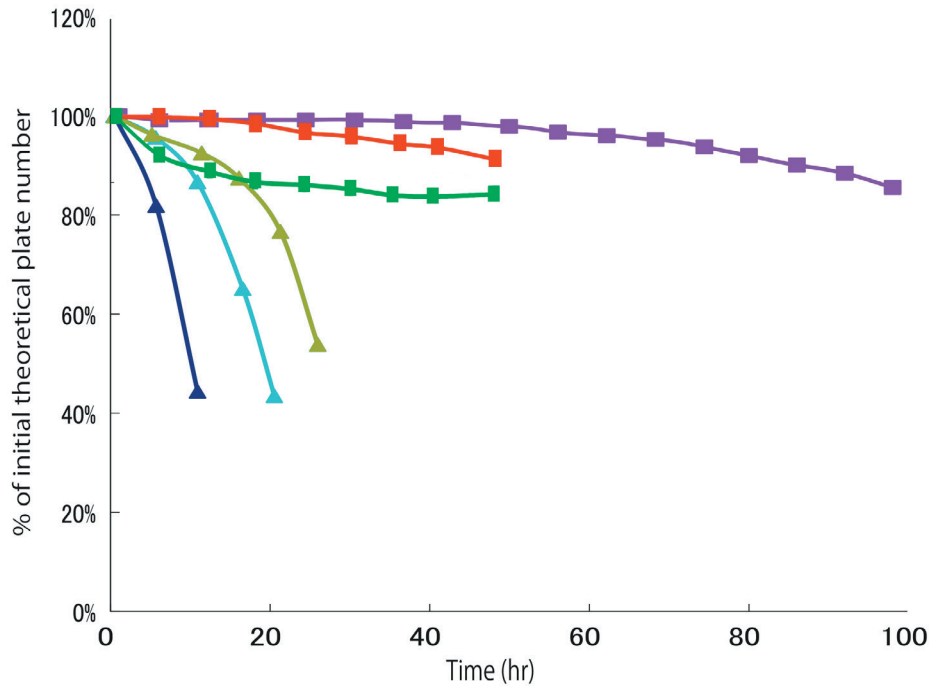


Figure 1:
Principle of ion-pairing chromatography

4. Requirements for the stationary phase

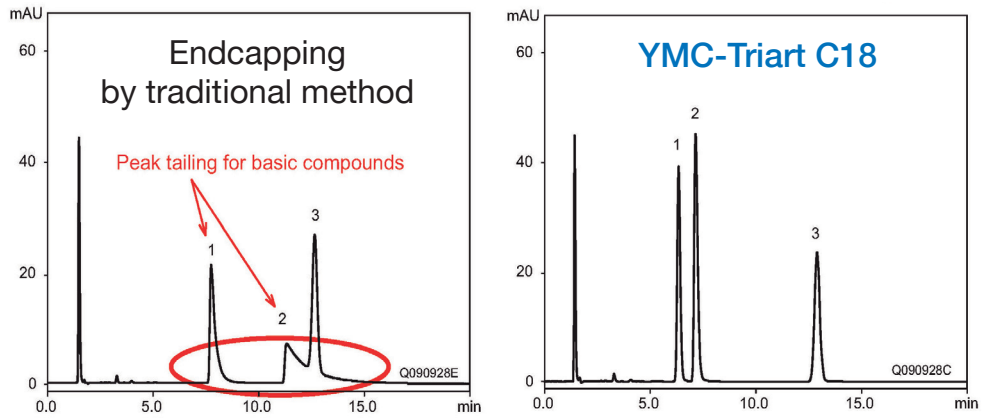
Considering the previously discussed issues, there are different requirements for the stationary phase. Working with the 2 pH unit rule often requires stationary phases which have a broad range of pH stability. Working at the extremes of pH values will cause chemical stress on the stationary phase which can lead to faster degradation and loss of column performance. At pH values above 6 dissolution of silica will occur. Modified silica-hybrid materials (e.g. YMC-Triart) have a wider pH stability range and are not easily hydrolysed at high or low pH. Figure 2 shows the pH stability of different stationary phases at pH 11.5 for up to 100 hours. For the YMC-Triart series the number of theoretical plates remains relatively constant with just a small decrease over the time, however for conventional phases – even for conventional hybrid based C18 – the plate count decreases very quickly.



Column:	YMC-Triart (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	TA12S05-1546PTH
Eluent:	50 mM K_2HPO_4-K_3PO_4 (pH 11.5)/methanol (90/10)
Flow rate:	1.0 mL/min
Temperature:	40 °C
Sample:	benzyl alcohol

Figure 2: pH stability of YMC-Triart and conventional phases

To minimise the activity of free silanols, the endcapping process is very important. The free silanols can interact with the analyte as described previously and therefore need to be endcapped. Traditionally, silica particles are single-endcapped after bonding of the modification phase (e.g. C18, C8) which is often not sufficient. This can be seen in Figure 3 on the left, since the peaks show obvious tailing when a traditional endcapped stationary phase is used. On the right, the analysis is shown using YMC-Triart C18 which is treated with a proprietary multi-stage endcapping process. Multi-stage endcapping ensures that the maximum amount of free silanols are shielded, which leads to significantly decreased tailing.



Ingredients in cough/cold medication:

1. Chlorpheniramine
2. Dextromethorphan
3. Propyl p-hydroxybenzoate

Column:	5 µm, 150 x 3.0 mm ID
Part No.:	TA12S05-1503PTH
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)/acetonitrile (65/35)
Flow rate:	0.425 mL/min
Temperature:	40 °C
Detection:	UV at 235 nm

Figure 3: Comparison of traditional and multi-stage endcapping

In summary, there are different techniques to prevent peak tailing successfully. The use of ion-pairing agents, pH adjustments and buffer considerations are helpful tools for obtaining a satisfying separation. This results in requirements for the stationary phase, such as high pH stability and an extensive endcapping process.