RP-Chromatography at elevated pH

Factors for success

Kromasil®

AKZO NOBEL

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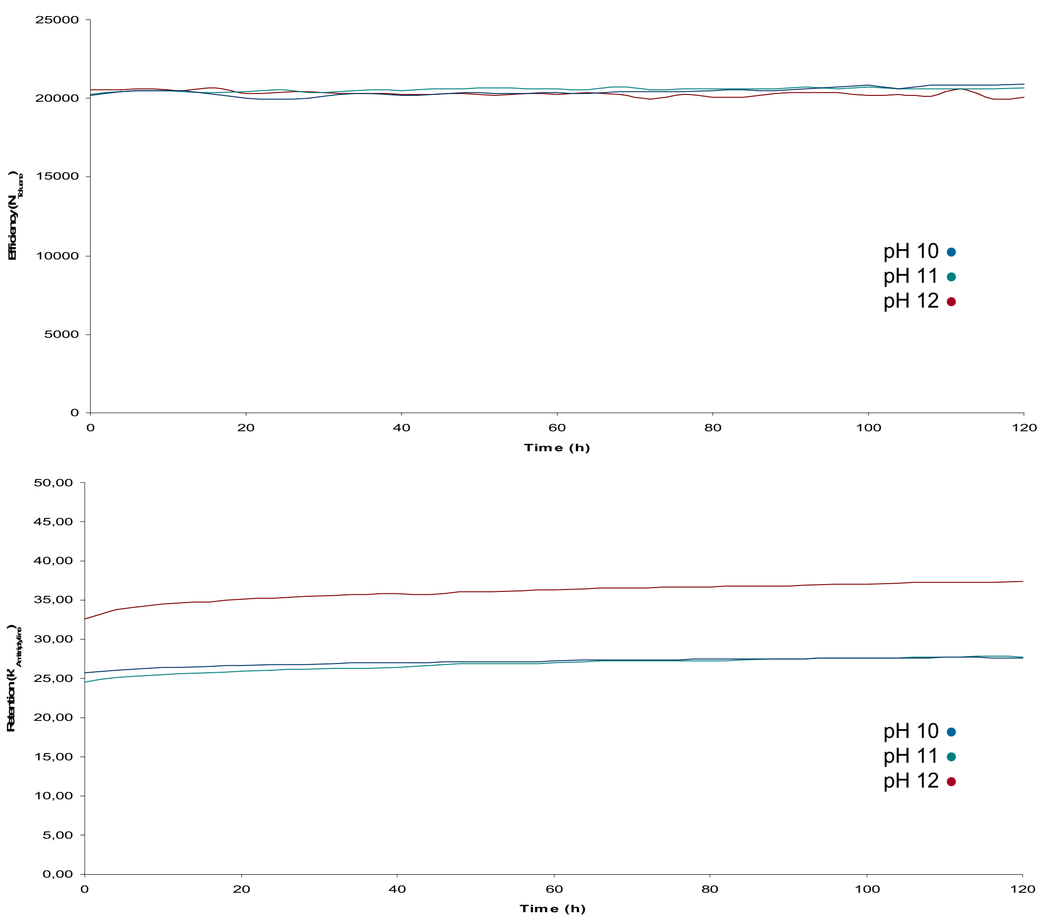
Introduction

The need to analyze and purify basic compounds is increasing. This creates a demand for silica based stationary phases to withstand long-term use at high pH. Performance and function of a Kromasil® C18 stationary phase have been evaluated during extended periods of time at elevated pH.

It is well known in reversed phase chromatography that the uncharged form of a compound is best suited for troubleless HPLC. Most basic drugs have pK_a values of around 9.5. To keep basic substances uncharged they must be kept in an environment with pH higher than its pK_a value. It is common practice to use a mobile phase pH that is up to two units higher that the pK_a value of the analyte. Our studies confirm that 0.5-1.0 unit above the target pK_a gives the best result both with respect to chromatography and off course lifetime of silica based stationary phase.

Chemical stability of Kromasil® C18 under alkaline conditions

Kromasil® RP stationary phases are very resistant to basic pH mobile phases. The results of long-term exposure of Kromasil® C18 to mobile phases with pH 10, 11, and 12 are seen in figure 1. Even after 120 hours of contact no theoretical plates were lost, and only minor changes in retention were observed.



Column: Kromasil® KR100-5-C18 250 x 4.6mm
Mobile phase: Acetonitrile/50mM buffer 50/50
Buffers: Trimethylamine/acetate pH 10.0
Triethylamine/acetate pH 11.0
Triethylamine/acetate pH 12.0
Flow rate: 1.0 ml/min during analysis
0.1 ml/min between analysis
Temperature: 25°C
Detection: UV@254 nm
Analytes: Toluene and Amitriptyline

Figure 1. Long term test of the chemical stability of Kromasil® C18 at alkaline pH

Factors for Success

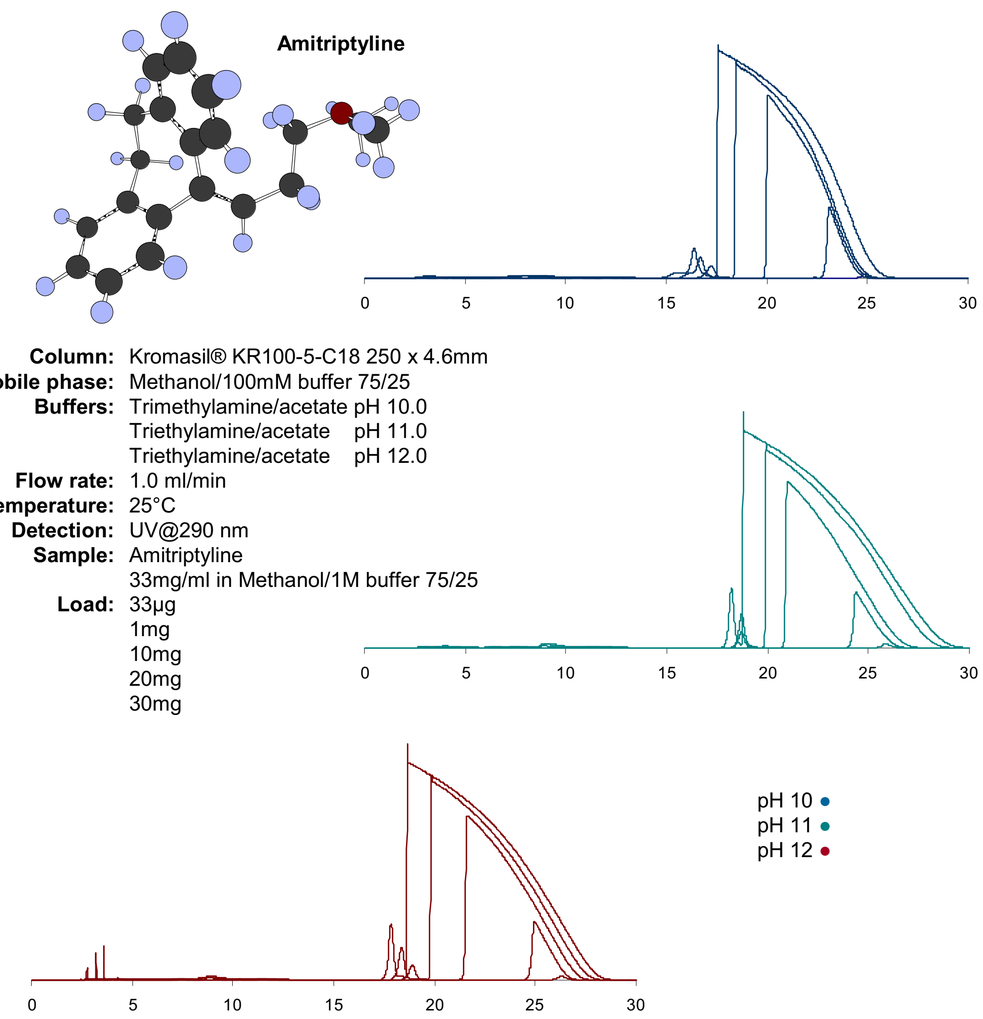
An intelligent choice of separation conditions will optimize both the separation and the lifetime of the HPLC column.
With a starting point in the pK_a of the target molecule a suitable pH can be chosen for the mobile phase. Hereafter an appropriate buffer should be chosen with the correct buffer zone. The selection of buffer will influence potential interface with MS instrumentation and work-up of prep pools.

The pH in the mobile phase should be about 0.5 to 1 unit higher that the pK_a in case of a basic analyte. In order to prolong the lifetime of the column and maintain the expected pH in a mobile phase mixed with an organic solvent, one should avoid phosphate and carbonate buffers[3]. These buffers exhibit significantly increased pH when mixed with organic solvent, as is often the case when preparing mobile phases. Left are different amines with buffer capacity in the basic region, these are also more easily removed after doing prep separations or during further analysis using mass spectrometry.

When doing preparative separations it is important that the feed has a high buffer capacity to buffer the target molecules before they enter the column, otherwise an instant peak splitting can occur due to the existence of different forms of the target molecule. This means that the feed should be prepared with a higher ionic strength than the mobile phase.

Overload experiments

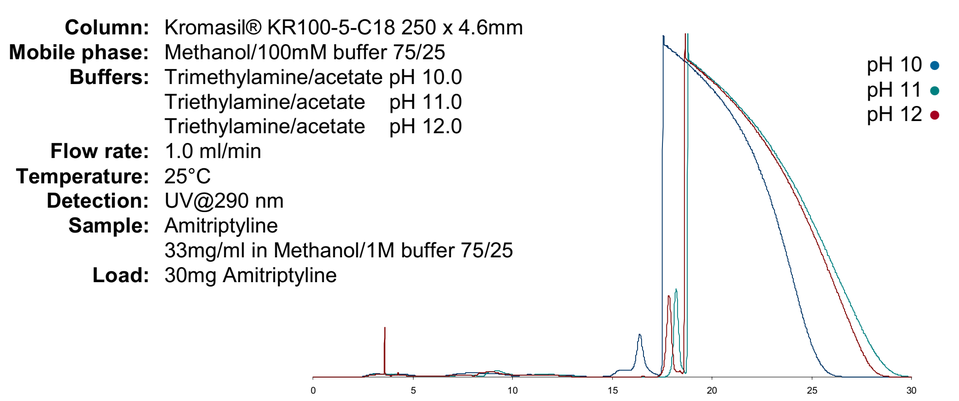
The overloading experiments are performed using amitriptyline with a pK_a of 9.4. Two organic buffers, trimethylamine and triethylamine, were chosen with pK_a values of 9.8 and 10.8 respectively. The chromatographic experiments are performed in mobile phases with pH 10, 11, and 12. Chromatograms were collected with different loads from analytical amounts up to 30mg using all three mobile phases and Kromasil® C18; the chromatograms are seen in figure 2.



Column: Kromasil® KR100-5-C18 250 x 4.6mm
Mobile phase: Methanol/100mM buffer 75/25
Buffers: Trimethylamine/acetate pH 10.0
Triethylamine/acetate pH 11.0
Triethylamine/acetate pH 12.0
Flow rate: 1.0 ml/min
Temperature: 25°C
Detection: UV@290 nm
Sample: Amitriptyline
33mg/ml in Methanol/1M buffer 75/25
Load: 33µg
1mg
10mg
20mg
30mg

Figure 2. Overloading experiments with amitriptyline on Kromasil® C18 using different mobile phase pH

In all three cases the overloading displays classical langmuirian triangular peaks, with the front moving forward as the load increases. The appearance of the chromatograms at pH 11 and 12 are almost identical, whereas the width of the overloaded peaks are narrower at pH 10. The peak width of the overloaded peaks determine the maximum loadability $C_{s\ max}$ of a stationary phase, meaning that the loadability of amitriptyline on Kromasil C18 appears to be higher at pH 10 than at pH 11 and 12. The chromatograms with 30 mg load at all three pH's are compared in figure 3.



Column: Kromasil® KR100-5-C18 250 x 4.6mm
Mobile phase: Methanol/100mM buffer 75/25
Buffers: Trimethylamine/acetate pH 10.0
Triethylamine/acetate pH 11.0
Triethylamine/acetate pH 12.0
Flow rate: 1.0 ml/min
Temperature: 25°C
Detection: UV@290 nm
Sample: Amitriptyline
33mg/ml in Methanol/1M buffer 75/25
Load: 30mg Amitriptyline

Figure 3. Comparison of overloaded chromatograms at pH 10, 11, and 12

From the comparison it is observed that not only the loadability but also the separation from the impurity eluting just before the amitriptyline peak is best at pH 10.

Conclusion

Based on the experiments presented in this poster it is concluded that a mobile phase with pH of about 0.5 to 1 unit above pK_a of the analyte is enough to ensure good chromatographic behavior of a basic drug in RP HPLC. Also it was verified that Kromasil® C18 phases tolerate the exposure to basic mobile phases up to pH 12 over extended periods of time, and they are therefore suited for separation of basic molecules in both analytical and preparative scale.

References

[1] Heinisch, S. Rocca, J.L., *J. Chromatogr. A,* (1048) **2004**, 183-193.
[2] Neue,U.D. et al., *J. Chromatogr. A*, (1030) **2004**, 123-134.
[3] Tindall, G.W. Perry, R.L., J. Chromatogr. A, (988) **2003**, 309-312.