Investigation of overloading of basic compounds in Ultra High Pressure Liquid Chromatography – Influence of the mobile phase pH

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Introduction

Liquid chromatography is a popular technique for pharmaceutical analysis and separations. The recent development of Ultra High Performance Liquid Chromatography (UHPLC) has led to better column efficiencies as well as shorter analysis times.
In the pharmaceutical industry most drugs are basic compounds which can lead to peak tailing and efficiency loss. The overloading phenomenon is also frequently observed.
The objective of this study was to highlight the overloading for a basic compound as a function of the mobile phase pH. Clozapine is an antipsychotic drug used in schizophrenia treatment. The aim of this work was to obtain the best operating conditions to obtain good peak shapes for basic compounds used in the pharmaceutical industry.

Experimental methods

UPLC:	Acquity Waters
Detection:	UV @ 220 nm
Column:	Acquity BEH 300 C18; 5cm*2.1mm*1.7µm
Flow rate:	0.5mL/mn
Buffer pH:	Ammonium Acetate (pH 6.8), Formic Acid (pH 2.7), Ammonia (pH 10.4)
Injected volume:	0.5 µL
Temperature:	30°C
Sample:	clozapine (5, 50, 100 and 200ppm)
Gradient:	

	Time (minutes)	Acetonitrile (%)	Water (%)
Initial	0	5	95
Gradient	1.7	80	20
Descent	2	5	95
Equilibration	3.5	5	95

Clozapine was analyzed at four concentrations in three buffers.

Results

The analyses showed peak deformation which resulted in both efficiency and retention time decrease when the injected sample mass is increased. The Clozapine peaks approximates to right-angled triangles as the concentration is raised, which is characteristic of overloading.
When the pH is higher, the asymmetry decreases and the peak shape is improved. Moreover the retention time difference between two concentrations becomes very small.
This phenomenon was explained in the literature by ion exchange interactions between stationary phase silanols (which are ionized at low acidic pH) and basic compounds.
Better results were obtained with intermediate pH such as ammonium acetate buffer (pH 6.8) because some columns got damaged with a higher pH such as ammonia buffer (pH 10.4).

Chromatogram obtained during overloading study

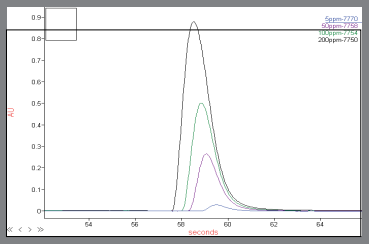

Figure 1: Chromatogram obtained with acid formic (pH 2.7)

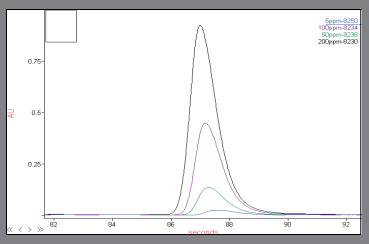

Figure 2: Chromatogram obtained with ammonium acetate (pH 6.8)

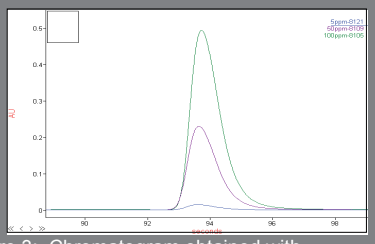

Figure 3: Chromatogram obtained with ammoniac (pH10.4)

Conclusion

Overloading is a complicated phenomenon which remains unclear, but the mobile phase pH is an important parameter which must be chosen in order to increase the column charge capacity.

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