Investigation into the stability of HILIC based Cetirizine assay

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Abstract

Cetrizine is a major metabolite of hydroxyzine, and a racemic selective H1 receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. The structural similarity of cetirizine to hydroxyzine, and its derivation from piperazine, attribute similar adverse reactions and properties to other piperazine derivatives. It is available over-the-counter in a wide range of countries.

A HILIC (Hydrophilic Interaction Liquid Chromatography) based separation mechanism is given as one of the USP methods for the analysis of cetirizine and its impurities. HILIC is renowned for instabilities in terms of retention times, and the amount of equilibration required for the chromatographic system.

Examples are given that demonstrate the unstable nature of some HILIC separations, concentrating on a specific USP method for the analysis of Cetirizine. Three parameters are varied for this assay and it will be demonstrated what effect the temperature, conditioning and the flow rate can have in terms of key chromatographic parameters.

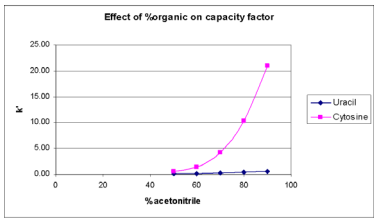
Introduction

When polar stationary phases such as silica are used in the presence of a high organic mobile phase such as acetonitrile, and a relatively small amount of aqueous mobile phase, a semi-immobilised aqueous layer can be formed on the surface of the stationary phase. The depth of this bound layer is affected by buffer concentration and also the amount of water in the bulk mobile phase. This water rich layer then acts to retain polar analytes through a liquid-liquid type separation mechanism, with the polar stationary phase interacting directly with the analyte to increase the retention further.

Consequently, HILIC is used to retain many highly polar compounds such as nucleotides, phosphorylated peptides and carbohydrates. Such compounds would pass, mostly unretained, through a hydrophobic column such as a C18.

In HILIC mode, the strength of the mobile phase is controlled by the amount of organic modifier used. If more organic is used in the mobile phase then greater retention is observed, which is the opposite of what is observed in reverse-phase chromatography. Figure 1 demonstrates this effect, as it can be seen that increasing the amount of ACN results in an increase in the capacity factor for both of the compounds under investigation as the HILIC effect becomes the dominant mode of interaction.

FIGURE 1. Effect of increasing the % organic on capacity factor



Column: Silica 5 µm, 150 x 4.6 mm

Mobile phase: 10mM Ammonium Acetate, pH 5.0 / MeCN

Flow rate: 0.6 mL/min

Detection: UV at 254 nm

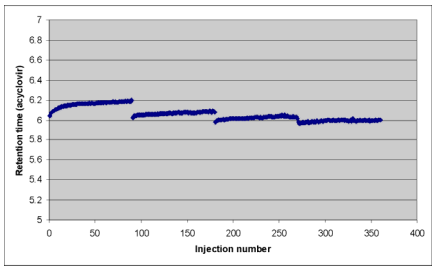
Temperature: 30 °C

Results

Sensitivity of HILIC Separations

One of the challenges associated with HILIC is that some compounds are very sensitive to very small changes in the mobile phase. Figure 2 demonstrates this effect. It can be clearly seen when a new mobile phase has been prepared, as the retention time for a polar compound (in this case acyclovir) changes. This can also be seen from Figure 1, where the gradient for the retention time increases substantially as the amount of organic is increased, suggesting that small differences in the composition of the mobile phase will have a significant effect on the retention of the compound.

FIGURE 2. Effect of making new mobile phase



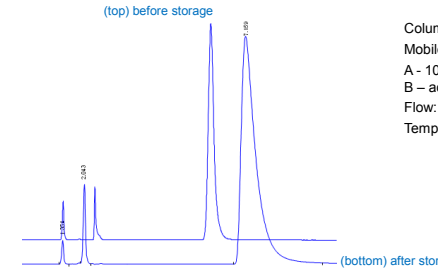
- Column: Polar bonded silica 5 µm, 150 x 4.6 mm
- Mobile phase:

50 mM Ammonium Acetate, pH 5.0 / MeCN (10:90)

- Flow rate: 0.6 mL/min
- Detection: UV at 254 nm
- Temperature: 30 °C

Conditioning and storage of the column is another experimental parameter that has been demonstrated to be of great importance in performing a HILIC separation and Figure 3 demonstrates this effect. The chromatography for Cytosine was initially developed on a silica column using HILIC mode of chromatography. The column was stored in water/acetonitrile (15:85) so that it was not left in a buffered solution. However , after re-equilibrating the column and starting the analysis again it can be clearly seen that the retention time has shifted and also the peak shape has deteriorated.

FIGURE 3. Effect of column storage



Column: Silica 5 µm 150x4.6mm

Mobile Phase (50:50):

A - 10mM ammonium acetate, pH 5.0

B – acetonitrile

Flow: 1.0 mL/min

Temperature: 25° C

Cetirizine assay

The experimental conditions used for the cetirizine assay investigated here were based on the USP method:

- Mobile phase: 93:6.6:0.4 ACN / Water / 1M Sulfuric Acid
- Flow rate: 1 mL/min; Detection: UV at 230 nm
- Injection: 20 µL
- Column: Hypersil Silica 5 µm, 250x4.0mm

FIGURE 4. Effect of conditioning of the column.

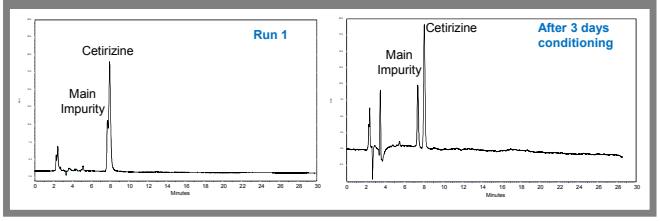


Figure 4 shows the effect that conditioning of the column can have in terms of resolution and also in terms of retention of the main peak and the impurity peak. The column was conditioned with mobile phase over a period of three days, and then the column was retested. It is clear that running a very well conditioned column improves the separation dramatically, and also increases the retention of both of the compounds.

Conditioning of the column, particularly if it is unmodified silica is very important as the surface can be prone to modifications in the presence of water due to hydrolysis interactions which will alter the retention mechanism.

The USP specifications for this method are based on the resolution of cetirizine and the major impurity, with this value being greater than 2. The aim of the experiments is to achieve the best possible resolution, to make the assay more robust.

FIGURE 5. Effect of altering temperature.

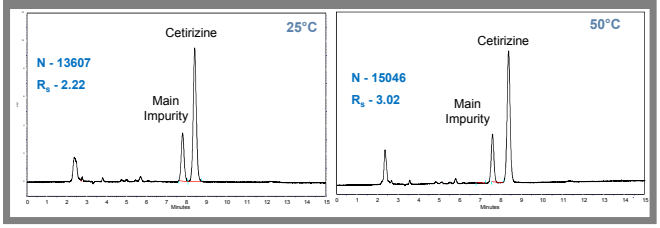


Figure 5 shows the effect of running the assay at 25°C and at 50°C. The performance of the assay improves as the temperature is increased with the resolution increasing from 2.2 to over 3 at the higher temperature. Increasing the temperature increases the resolution between the major components and it also increases the efficiency of the main cetirizine peak.

FIGURE 6. Effect of optimising flow rate.

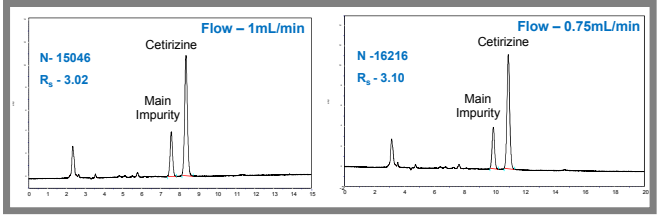


Figure 6 shows the effect of altering the flow rate. It can be seen that lowering the flow rate has some effect on the efficiency increasing it from 15,000 plates per metre to over 16,000 plates per metre. This results in a minor increase in the resolution, as this parameter is proportional to the square root of efficiency. The reason for lowering the flow rate was that the specified column diameter was 4.0mm, and the flow of 1.0 mL/min is the nominal optimum value for a column which has an internal diameter of 4.6 mm. Thus, the flow rate was scaled down accordingly to account for the slight difference in internal diameter.

Discussion

Silica columns can suffer from changes to the surface morphology in the presence of water, due to hydrolysis of the hydroxyl groups. This is most prone to happen when using them in HILIC mode where the adsorption of an aqueous layer is required for the separation to be achieved. Thus small changes in storage/conditioning conditions, mobile phase composition can have a substantial effect on the retention mechanism, and ultimately the chromatography. Using the parameters discussed here it is possible to make the chromatography more robust.

Conclusion

HILIC chromatography has been show to be an ideal tool for the separation scientist, however understanding the stability of the assay is key to making the technique work effectively. The data presented here demonstrates the importance of:

- Temperature
- Mobile phase composition
- Column equilibration
- Flow rate

All of these parameters should be optimised before method development is complete.

Additional Information

For additional information, please visit our Chromatography Resource Centre which can be found at:

www.thermoscientific.com/chromatography

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