Core-Shell Particle Column Performance in Acetylsalicylic Acid Related Substances Testing by Rapid Resolution Liquid Chromatography

Christine Cassar [1], Hans Helmut Frey [2] and Claude Farrugia [1]

[1] Department of Chemistry, University of Malta, Msida MSD2080, Malta

[2] Development Division, Actavis Ltd., Bulebel Industrial Estate, Zejtun ZTN3000, Malta

Introduction

Until recently, HPLC column performance was improved by shortening the column and decreasing the particle size below the 2-µm limit. A significant disadvantage of this technique is ultra high back pressures. Recently developed Kinetex® core-shell columns have been designed to achieve sub-2-µm efficiency without the ultra high back pressures by diffusing the mobile phase through the small volume of the stationary phase, the shell. The objective of this study was to investigate the suitability of core-shell column technology for the related substances testing of acetylsalicylic acid using rapid resolution liquid chromatography (RRLC).

Methodology

The European Pharmacopoeial related substances test for acetylsalicylic acid was applied on RRLC apparatus, using a Phenomenex® Kinetex 2.6 µm C18 50 × 4.6 mm core-shell column. The method parameters altered were the flow rate, temperature and injection volume. In the first part of the study, a one variable at a time (OVAT) approach was adopted, changing one parameter at a time while keeping the other parameters constant. In the second part of the study, a full factorial approach was adopted and parameters were changed concurrently to reveal any synergistic effect. The influence of changes in the method was determined by statistical analysis of the retention time, peak area, theoretical plates, asymmetry and resolution.

Results

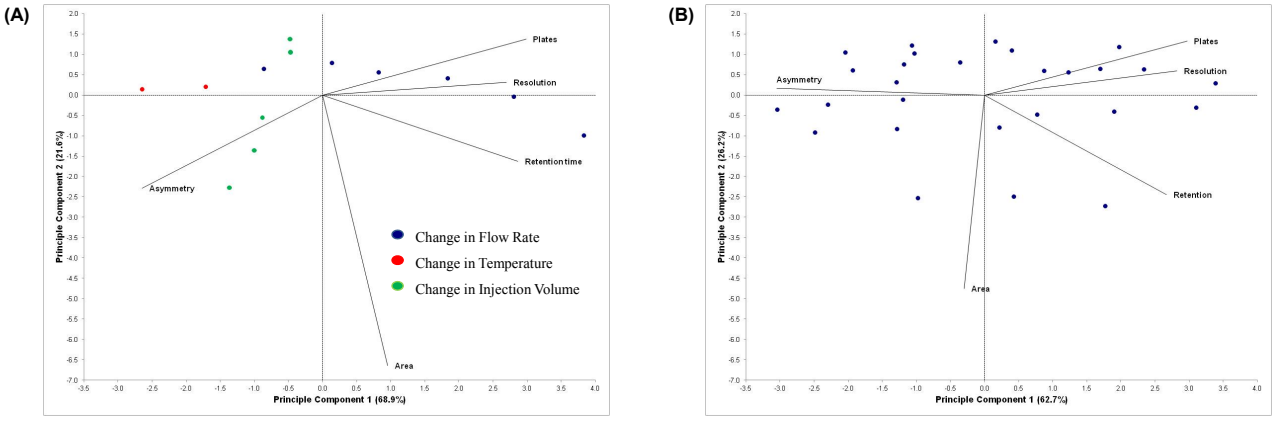

Figure 1: Principle component analysis (PCA), showing the correlation between the dependent variables under different experimental conditions using the (A) OVAT and (B) full factorial approach.

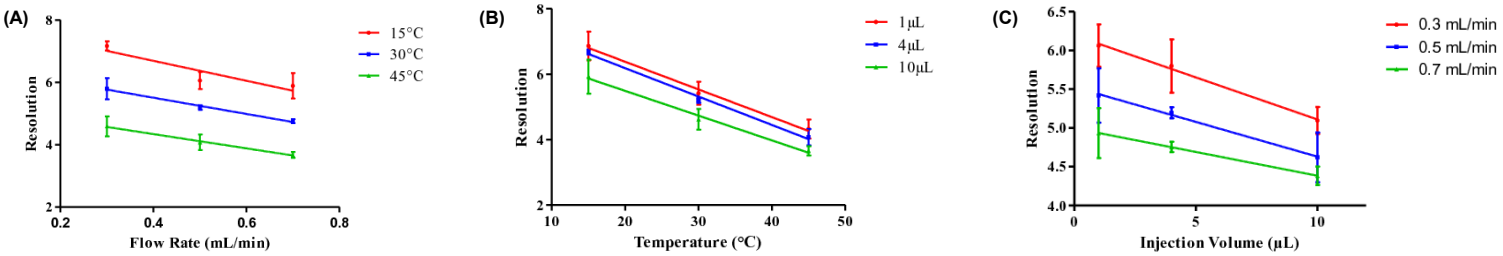

Figure 2: Graphs illustrating the effect on resolution between the acetylsalicylic acid and salicylic acid peaks of (A) increase in flow rate at different temperatures with an injection volume of 4 µL, (B) increase in temperature at different injection volumes with a flow rate of 0.5 mL·min^{-1}, and (C) increase in injection volume at different flow rates with a temperature of 30 °C.

Conclusions

Resolution: The resolution between the acetylsalicylic acid and salicylic acid peaks decreased as the flow rate, temperature and injection volume increased. However, the rate of decrease and resolution values differed from one variable to the next. Optimal resolution was achieved at low flow rates, temperature and injection volume. As retention time and theoretical plate number increased, the resolution improved.

Retention Time: The retention time decreased with increasing flow rate up to a maximum of approximately 0.5 mL/min, following which the retention time did not change significantly.

Peak Area: This was amplified at low flow rates and high injection volumes, the latter having a larger influence on the peak area.

Theoretical Plate Number: The plate count, which is a measure of efficiency, decreased at high flow rates and injection volumes, and exhibited an inverse relationship with asymmetry.

Comparison with the fully-porous column: Resolution was substantially higher in the fully porous column[1] than in the core-shell column. A longer and fully-porous column provides longer pathway and hence the retention time is delayed. The core-shell structure restricts the pathway only to the shell. If the retention time is shortened, the analysis time can be reduced and so decrease the cost for analysis. Hence, the core-shell column is more economical.

References

1. Borg, D.A. A Preliminary Study for the Method Transfer of Acetylsalicylic Acid from HPLC to RRLC. B.Sc.(Hons.) Dissertation, 2009.

Acknowledgements

The authors thank Actavis Ltd. for the supply of chemicals, and for the access to scientific equipment in the laboratories of the company.