



Optimization of ion-suppression reverse phase-separation of basic compounds using different mobile phases



Gabriela Petrovska-Dimitrievska^{1*}; Jelena Acevska¹; Marija Zafirova Gjorgievska¹; Liljana Ugrinova¹; Natalija Nakov¹; Vasil Karcev¹; Aneta Dimitrovska¹; Katerina Brezovska¹

¹Faculty of Pharmacy, Un. "Ss Cyril and Methodius", Mother Theresa 47, 1000 Skopje, Republic North Macedonia

INTRODUCTION

The aim of this study was to monitor the chromatographic behavior of two basic compounds (pK_a (analyte 1) = 10.89 and pK_a (analyte 2) = 8.32) when the composition of the mobile phase varies, regarding: composition of the buffer solution, pH value of the mobile phase and percentage of the organic solvent (acetonitrile). The optimal experimental conditions were selected using design of experiments (DoE).

MATERIALS AND METHODS

- Shimadzu UHPLC Nexera, with DAD detector;
- C18 Acclaim Polar Advantage II 120 A, 250 mm x 4.6 mm; 5 μ m;
- Lichrospher RP B-select 125 mm x 4.0 mm; 5 μ m;
- Seven Multi pH meter, Mettler Toledo;
- MODDE 10.0 Software (Umetrics, Sweden).

RESULTS AND DISCUSSION

Sodium acetate buffer

The 2² Full Factorial DoE suggested conduction of 11 experiments, using different pH values of sodium acetate buffer (0.05 M sodium acetate, pH values: 3.5; 4.0; 4.5), with variable amount of acetonitrile (50%, 60% and 70%) in the mobile phase. Best separation of the analytes (resolution, peak symmetry and theoretical plates, reasonable retention time) was achieved using mobile phase with the lowest pH value of the aqueous buffer and the maximum concentration of acetonitrile (70% v/v).

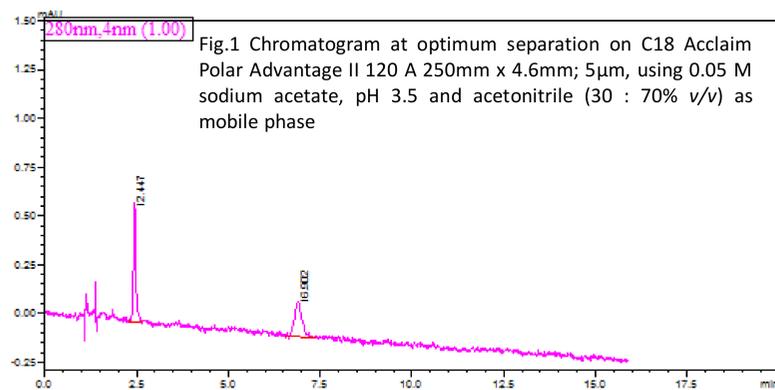


Fig.1 Chromatogram at optimum separation on C18 Acclaim Polar Advantage II 120 A 250mm x 4.6mm; 5 μ m, using 0.05 M sodium acetate, pH 3.5 and acetonitrile (30 : 70% v/v) as mobile phase

Potassium dihydrogen phosphate buffer

The pH value of this buffer was adjusted using 85% phosphoric acid to obtain the values for pH 3.0 and 3.5. Acetonitrile was used in the mobile phase in two different amounts (65% and 70% v/v). The lowest pH of the aqueous buffer (3.0) combined with 65% v/v of acetonitrile was selected as the most optimal mobile phase for separation of tested basic compounds (pH =4.0).

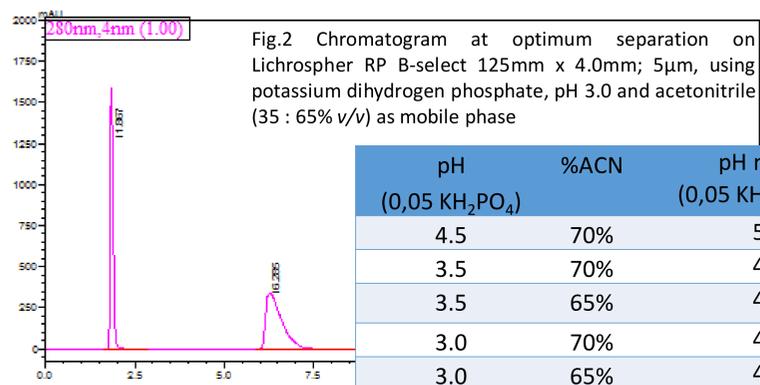


Fig.2 Chromatogram at optimum separation on Lichrospher RP B-select 125mm x 4.0mm; 5 μ m, using potassium dihydrogen phosphate, pH 3.0 and acetonitrile (35 : 65% v/v) as mobile phase

Citric acid / Disodium hydrogen phosphate buffer

The different combinations of buffers composed of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate cover wide range of pH values (2.5-7.5). Using 2² Full Factorial DoE, three pH values for the buffer mixtures (2.5; 5.0; 7.5) in combination with acetonitrile (30%, 60% and 90% v/v) as an organic solvent fraction were evaluated. (Fig.3 and Fig 4). The most optimal conditions for the separation of basic compounds were achieved using buffer combination with pH=2.5 and 70% v/v of the organic solvent (pH value of mobile phase was 3.63) on Lichrospher RP B-select column.

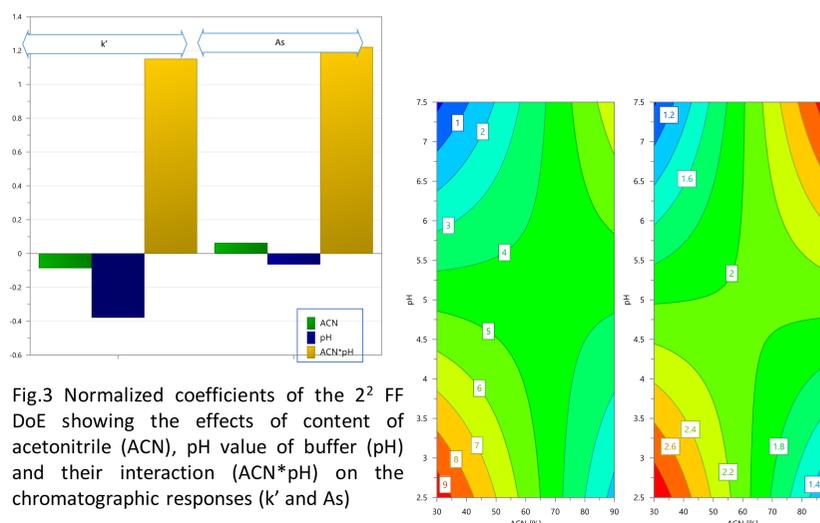


Fig.3 Normalized coefficients of the 2² FF DoE showing the effects of content of acetonitrile (ACN), pH value of buffer (pH) and their interaction (ACN*pH) on the chromatographic responses (k' and A_s)

Fig.4 Response surface of the influence of the acetonitrile content and pH value of the buffer on the retention factor (k') and asymmetry factor (A_s)

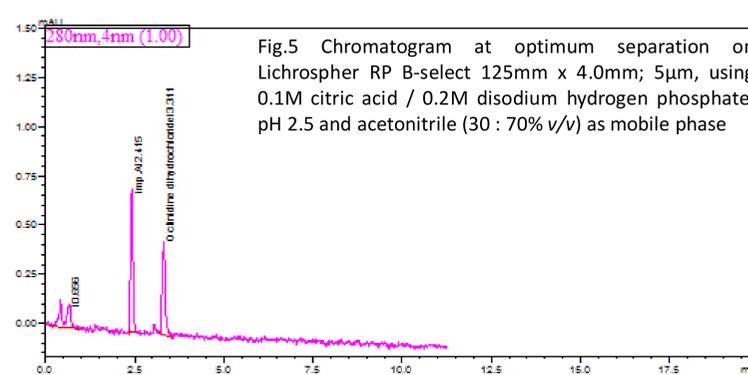


Fig.5 Chromatogram at optimum separation on Lichrospher RP B-select 125mm x 4.0mm; 5 μ m, using 0.1M citric acid / 0.2M disodium hydrogen phosphate, pH 2.5 and acetonitrile (30 : 70% v/v) as mobile phase

CONCLUSION

Different sets of experiments were conducted for optimization of ion-suppression reverse-phase chromatography of polar basic compounds, including selection of column, pH value of the buffer and the percentage of the organic solvent in the mobile phase. Optimal retention times of the two basic compounds and satisfactory elution was achieved on both C18 and C8 columns, optimized for separation of basic compounds. The results have shown that the shift of the pH values due to different percentage of acetonitrile in the mobile phase, depends on the type and properties of the buffering system, the amount of the organic solvent, starting pH value of aqueous buffer and concentration of the buffer. Lowering the amount of acetonitrile and increasing the pH value of the aqueous mobile phase increase the retention time, due to reduced degree of ionization of the basic compounds, influencing the selectivity and robustness of the method.

References

- Bosch, E., Espinosa, S., Roses, M., 1998. Retention of ionizable compound in high-performance liquid chromatography III Variation of pK values of acid and pH values of buffers in acetonitrile-water mobile phase, *J. Chromatogr. A*, 824, 137-146.
- Canals, I., Valko, K., Bosch, E., Hill, A.P., and Roses, M., 2001. Retention of Ionizable Compounds on HPLC. 8. Influence of Mobile-Phase pH Change on the Chromatographic Retention of Acids and Bases during Gradient Elution, *Anal. Chem.* 73, 4937-4945.
- Espinosa, S., Bosch, E., Roses, M., 2002. Retention of ionizable compounds in high-performance liquid chromatography 14. Acid-base pK values in acetonitrile-water mobile phases, *J. Chromatogr. A*, 964, 55-66.
- Gritt, F., Guiochon, G., 2004. Retention of ionizable compounds in reversed-phase liquid chromatography. Effect of the ionic strength of the mobile phase and the nature of the salt used on the overloading behavior, *Anal. Chem.* 76, 4779-4789.
- Heinisch, S., Rocca, J.L., 2004. Effect of mobile phase composition, pH and buffer type on the retention of ionizable compounds in reversed-phase liquid chromatography: application to method development, *J. Chromatogr. A*, 1048, 183-193.
- Heinisch, S., Puy, G., Barrioulet, M.P., Rocca, J.L., 2006. Effect of temperature on the retention of ionizable compounds in reversed-phase liquid chromatography: Application to method development, *J. Chromatogr. A*, 1118, 234-243.
- Subirats, X., Roses, M., Bosch, E., 2007. On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the pK_a of Analytes - a Review, *Separation & Purification Reviews*, 36:3, 231-255.