

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

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Introduction

The optimization of the method for separation of basic compounds using ion-suppression reverse-phase liquid chromatography, requires control of the selection of stationary phase and mobile phase composition: percentage of organic solvent, ionic strength of the buffers used and pH value (Heinisch and Rocca, 2004). The type and percentage of the organic solvent added in aqueous buffers with defined pH value, influence the pH value of the mobile phase, which affects the degree of ionization of basic compounds. The pKa values of the analyte and the pH value of mobile phase can be used to predict the degree of ionization of basic compounds in the mobile phase (Espinosa et al., 2002). Variation in the degree of ionization results in significant change in chromatographic retention and selectivity, which is more evident in cases where the pH value of mobile phase, is near the pKa value of the basic compound. Therefore, the deviations in the pH value can result in significant changes of the retention time (Bosch et al., 1998; Subirats et al., 2007). Two analytes with similar structure and small differences in their pKa values, can be successfully separated, by controlling the pH value of the mobile phase (Espinosa et al., 2002). The aim of this study was to monitor the chromatographic behavior of two basic compounds (pKa (analyte 1) = 10.89 and pKa (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

Separation of the basic compounds was performed on Shimadzu UHPLC Nexera, equipped with diode array detector, using C18 Acclaim Polar Advantage II 120 A 250 mm x 4.6 mm; 5 µm for the mobile phase containing sodium acetate (pH=3.5-6.5) and Lichrospher RP B-select 125 mm x 4.0 mm; 5 µm for the mobile phases containing citric acid in combination with disodium hydrogen phosphate (pH = 2.5 - 7.5) and potassium dihydrogen phosphate (pH = 4.5). Acetonitrile (50% to 90% v/v) was used as the organic solvent. The pH values of each buffer and of the prepared mobile phases were measured on Seven Multi pH meter (Mettler Toledo). Optimization of experimental factors was done by appropriate factorial design, using MODDE 10.0 Software (Umetrics, Umea, Sweden).

Results and discussion

Sodium acetate buffer

The Placket Burman 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. A shift towards higher pH values for each combination of buffer and organic solvent (pH = 5.2-5.8), compared to the pH values of the buffers alone, was detected (pH =3.5 - 4.5). C18 Acclaim Polar Advantage II column, is a polar embedded column, which provides satisfactory separation of basic compounds. The experiments showed that best separation of the analytes (resolution, peak symmetry and theoretical plates) within reasonable retention time of less than 15 minutes, was achieved using mobile phase with the lowest pH value

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(5.2) of the aqueous buffer and the maximum concentration of acetonitrile (70% v/v).

Citric acid / Disodium hydrogen phosphate buffer

Different combinations of buffers composed of citric acid 0.1M and disodium hydrogen phosphate 0.2 M covering a wide range of pH values (2.5-7.5) were used (Merck Buffer Reference Center). Using the 2² Full Factorial DoE, three pH values for the buffer mixtures (2.5; 5.0; 7.5) were selected, in combination with acetonitrile (30%, 60% and 90% v/v) as an organic solvent fraction. Taking into account the greater buffer capacity of the citrate/phosphate buffers, the pH value of the mobile phases showed different behaviour, compared with the pH values of the mobile phases containing acetate buffer. The range of the measured pH values of the mobile phases was within pH=3.0-5.8. The variations of the pH values of mobile phase with different portions of acetonitrile, generate different degree of ionization of the analytes. This explains the difference of the retention times of the analytes, observed in the chromatograms, when different combinations of mobile phases were used. In the column used, Lichrospher RP B-select, the starting silica material is optimized for prevention of any secondary interactions with basic compounds, providing satisfactory separation without the need of use of ion-pair reagent. The most optimal conditions were achieved using buffer combination with pH=2.5 and 70% v/v of the organic solvent (pH value of the mobile phase was 3.63).

Potassium dihydrogen phosphate buffer

The initial pH value of the aqueous buffer potassium dihydrogen phosphate was 4.5. 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 concentrations (65% and 70% v/v). The pH values of the prepared mobile phases showed shift to higher values, similarly to the mobile phases containing acetate buffer (4.0-5.7). The values of system suitability parameters have confirmed the efficacy of the used column. 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).

Conclusion

In this study, a 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.

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