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Impact of mobile phase composition on reverse-phase separation of polar basic compounds

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Abstract

Method development for determination of polar basic compounds on ion-suppression reverse-phase liquid chromatography, requires appropriate selection of stationary phase and composition and pH value of the mobile phase. The addition of the organic solvent in aqueous buffers with defined pH value, results in shift of the pH value of the mobile phase, resulting in significant change in chromatographic retention and selectivity. The aim of this study was to evaluate the impact of the variation of pH value of the mobile phase and percentage of the organic solvent, on the chromatographic separation of basic compound (octenidine dihydrochloride). The design of experiment was used for the optimization of the chromatographic conditions and selection of the most suitable mobile phase composition. The optimal composition of the mobile phase, providing satisfactory chromatographic performance based on ion-suppression reverse-phase chromatography, included 0.1M citric acid/0.2M sodium dihydrogen phosphate, pH =2.5, combined with 70% v/v organic solvent-acetonitrile. The suitability of the method was confirmed by satisfactory results for the system suitability parameters, specificity, linearity and precision.

Key Words: polar basic compounds, ion-suppression, pH value, composition of mobile phase

Introduction

Reverse-phase separation of polar basic compounds is commonly achieved using ion-pair chromatography or by suppressing ionization, by adjusting the pH value of the mobile phase according to the pKa value of the analyte. A number of analytical columns for reverse-phase

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chromatography with silica based packing material have been optimized to provide satisfactory separation of polar basic compounds (Euerby and Patersson, 2003; Stella et. al., 2001.). Therefore, method development for determination of polar basic compounds on reverse-phase liquid chromatography (RP-LC) without ion pair reagents requires strict control of selection of stationary phase and mobile phase: percentage of organic solvent, ionic strength of the buffers used and pH value. pH value influences the degree of ionization of the analyte, whereas mobile phase composition (type and percentage of the organic modifier and type and ionic strength of the buffer) determines the retention of the ionized analyte (Rocca, 2004). The addition of the organic solvent (e.g. acetonitrile) in aqueous buffers with defined pH value, results in shift of the pH value of the mobile phase. This change affects the degree of ionization of basic compounds, resulting in significant change in chromatographic retention and selectivity. 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 used for chromatographic separation. In cases where the pH value of mobile phase, is near the pKa value of the basic compound, the deviations in the pH value can result in significant changes of the retention time (Roses et. Al., 2002).

The aim of this study was to monitor the chromatographic behavior of basic compound (octenidine dihydrochloride) with the variation of pH value of the mobile phase and percentage of the organic solvent, using ion-suppression reverse-phase chromatography. Octinidine dihydrochloride (N-octyl-1-[10-(4-octyliminopyridin-1-yl)decyl]pyridin-4-imine; dihydrochloride), is a cationic surface compound with antimicrobial effect. It contains two non-interactive cation centers in its structure, which are separated by a long aliphatic hydrocarbon chain (Assadian, 2016). Design of Experiments (DoE) will be used for the exploring the retention behavior and peak shape of octenidine, which would facilitate the optimization of the chromatographic conditions and selection of the most suitable mobile phase composition.

Materials and methods

Materials

Octenidine dihydrochloride (purity GC, 99.1 %,) product number: H62257, Lot No. Q29H018, (Thermo Fisher Scientific).

Reagents

Acetonitrile (HPLC grade), Sodium acetate trihydrate (for analysis ISO-ACS), Acetic acid, glacial (99,0-100,5 %, for analysis), Sodium hydroxide (for analysis ISO-ACS), Citric acid (for analysis ISO-ACS reagent Ph.Eur.), Disodium hydrogen phosphate (for analysis ISO-ACS reagent Ph.Eur.), Potassium dihydrogen phosphate (for analysis ISO-ACS reagent Ph.Eur.) and Phosphoric acid 85% (for analysis) were purchased from Merck, Darmstadt, Germany. Water (highly purified) was obtained with a TKA-LAB Reinstwasser system (Niederelbert, Germany).

Apparatus

HPLC Rapid Resolution Agilent 1200 Series: Micro Vacuum Degasser (G1379B), Binary Pump (G1312B), Well Plate Autosampler (G1367C), Thermostated Column Compartment (G1316B), Diode Array Detector (G1315C) and software Agilent OpenLab Intelligents Reporting C.0107SR1 [113]; Multi pH meter (Mettler Toledo); MODDE 10.0 Software (Umetrics, Umea, Sweden).

Chromatographic Conditions

Elution was performed on Lichrospher B-selected C8 125 mm x 4.0 mm; 5 μ m (MERCK). Mixture of 0.1M citric acid/0.2M sodium dihydrogen phosphate, pH =2.5, combined with 70% v/v of acetonitrile (pH value of the mobile phase is 3.63) was used as mobile phase, at a flow rate of 1.0 mL/min. The column temperature was maintained at 40°C. The injection volume was 5 μ L and detection was performed at 280 nm.

Preparation of solutions

10.0 mg (accurate weight) of octenidine dihydrochloride was transferred to a 25.0 mL volumetric flask, dissolved and diluted with mobile phase to volume (stock solution). The solution at working concentration (20 μ g/mL) was prepared by diluting 1.0 mL of the stock solution to 20.0 mL, with mobile phase.

The suitability of the method was checked by of specificity, linearity, precision and system suitability according to the ICH guideline for method validation (ICH Q2(R1)). Solutions were prepared by diluting the stock solution with mobile phase to obtain the required concentration range for corresponding parameters.

Results and discussion

Optimization of the method

Lichrospher RP B-select column was chosen as a suitable stationary phase for determination of octenidine dihydrochloride as a polar basic compound, taking into account that the starting silica material of this column is optimized in order to prevent any secondary interactions with basic compounds, therefore providing satisfactory separation without use of ion-pair reagent. Design of experiments (2^2 Full Factorial DoE), was used to explore the effect of mobile phase composition on retention behaviour (retention factor k') and peak shape (asymmetry factor, A_s) of octenidine dihydrochloride. The experimental variables comprised different combinations of buffers composed of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate, covering a wide range of pH values (2.5-7.5) with addition of acetonitrile (30%, 60% and 90% v/v) as an organic solvent fraction (Table 1). The range of the measured pH values of the mobile phases with different portions of acetonitrile varied within pH=3.0-5.8.

Table 1

The effects of the different chromatographic conditions on the defined chromatographic responses (retention behavior and peak shape) are shown through the normalized coefficients of the DoE (Fig. 1). The content of acetonitrile in the mobile phase does not have significant effect on the chromatographic responses, but the effect is in opposite ways for the retention (the k' is lower with higher content of acetonitrile) and symmetry (higher content of acetonitrile improve symmetry) of octenidine. Similarly, the pH value of the buffer used for preparation of the mobile phase have little effect on symmetry, but is considerably important for the retention factor (octenidine is more retained on column when the pH is lower). However, the major impact on both chromatographic responses (k' and A_s) is seen with the combination of both experimental variables. This interaction would not so easily recognized if the optimization was done by exploring one-factor-at-time approach and goes in line with the literature data that acetonitrile significantly affects the pH value of the mixtures with buffers used for mobile phase. This was also confirmed by measuring the pH value of the acetonitrile/buffer mixtures (Table 1).

Experiments have shown that increase of pH value influence the peak shape (in experiments N4 and N5, the peaks are distorted and splitted), regardless the change of

concentration of the organic solvent. In the experiment N3, due to the low concentration of acetonitrile, the peak is not eluted from the column, which is the same situation in the experiment N1, where at low pH value the retention time of the peak is higher than 55 minutes. Suitable retention times and peaks shapes were obtained only in the experiments N2, demonstrating what is clearly indicated with the response surface of the DoE (Fig. 2).

Fig. 1

Fig. 2

As optimal chromatographic conditions for both response factors (retention and peak shape), 0.1M citric acid/0.2M sodium dihydrogen phosphate, pH =2.5, combined with 70% v/v of acetonitrile (pH value of the mobile phase is 3.63) was selected, taking into account the low solubility of octenidine dihydrochloride in acetonitrile.

Satisfactory system suitability requirements of the optimized method were obtained in total analysis time of 6 minutes. The obtained values from system suitability tests (Table 2) for number of theoretical plates (N), retention factor (k'), symmetry factor (A_s) and relative standard deviation (RSD %), indicate on a satisfactory column efficiency and adequate performance of the chromatographic system (Table 2).

Table 2

Specificity of the method was confirmed from the chromatograms obtained after application of solvent and standard solution of octenidine dihydrochloride (Fig. 3).

Fig. 3

The linearity of the method was confirmed in the concentration range from 14 $\mu\text{g/mL}$ - 26 $\mu\text{g/mL}$ (corresponding to 80%, 90%, 100%, 110%, 120%) using the regression analysis of the relationship between the response (peak area) and the concentration ($y = 21.869x - 0.1747$, $R^2 = 0.9996$).

The precision of the method was assessed at three different concentrations of octenidine dihydrochloride: 14 $\mu\text{g/mL}$ (RSD = 0.12 %), 20 $\mu\text{g/mL}$ (RSD = 0.27 %) and 26 $\mu\text{g/mL}$ (RSD = 0.57 %), corresponding to 80, 100, and 120% of the working concentration.

The obtained results from determination of specificity, linearity, precision and system suitability are in accordance with the requirements of the ICH guideline for method validation (ICH Q2(R1)).

Conclusion

In this study, using the design of experiment, the impact of the variation of pH value of the mobile phase and percentage of the organic solvent on the chromatographic separation of basic compound (octenidine dihydrochloride) was evaluated. The increase of pH value influence the peak shape, regardless the change of concentration of the organic solvent. At low pH values, the concentration of the organic solvent is crucial for the retention of the analyte. The optimal composition of the mobile phase, providing satisfactory chromatographic performance based on ion-suppression revers-phase chromatography, included 0.1M citric acid/0.2M sodium dihydrogen phosphate, pH =2.5, combined with 70% v/v of acetonitrile. The suitability of the method was confirmed by satisfactory results for the system suitability parameters, specificity, linearity and precision.

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Резиме

Влијание на составот на мобилната фаза врз разделување на поларни базни соединенија на реверзно-фазна течна хроматографија

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Клучни зборови: поларни базни соединенија, јонска супресија, рН вредност, состав на мобилна фаза

Развојот на метод за определување на поларни базни соединенија со јонска-супресија на реверзно-фазна течна хроматографија, бара соодветен избор на стационарна фаза како и состав рН вредност на мобилната фаза. Додавање на органски растворувач во водени пуфери со дефинирана рН вредност, води до поместување на рН вредноста на мобилната фаза, што резултира со значителна промена на ретенционото време и селективноста на методот. Целта на оваа студија беше да се определи влијанието на варијацијата на рН вредноста на мобилната фаза и процентот на органскиот растворувач, врз хроматографското разделување на базни соединенија (октенидин дихидрохлорид). За оптимизација на хроматографските услови и избор на најсоодветен состав на мобилната фаза беше употребен дизајн на експерименти. Оптималниот состав на мобилната фаза, кој овозможува задоволителни хроматографски перформанси на принцип на јонска-супресија на реверзно-фазна хроматографија, вклучуваше 0,1М лимонска киселина/0,2М натриум дихидроген фосфат, со рН =2,5, во комбинација со 70% v/v органски растворувач-ацетонитрил. Соодветноста на методот беше потврдена со задоволителни резултати за параметрите од соодветноста на системот: специфичност, линеарност и прецизност.

Table 1. Set of experimental conditions for method optimization used in the 2^2 Full Factorial DoE

Number of experiments	pH 0.1M Citric acid/0.2M Na ₂ HPO ₄	%ACN	pH of mobile phase (mixture)	RT of OCT
N1	2.5	30%	3.05	> 55 min
N2	2.5	90%	4.00	5.129 min
N3	7.5	30%	5.35	0.1*
N4	7.5	90%	5.60	14.693 min
N5	5.0	60%	5.75	11.413 min
N6	5.0	60%	5.75	11.413 min
N7	5.0	60%	5.75	11.413 min

* Within dead volume of the column

Table 2. System suitability values for the optimized method

System suitability parameter	Octenidine dihydrochloride
Rt (min)*	3.453
k'	2.8
As	1.2
N	5115
RSD _{Rt} (%)	0.05
RSD _{Peak area} (%)	0.27

$n = 6$

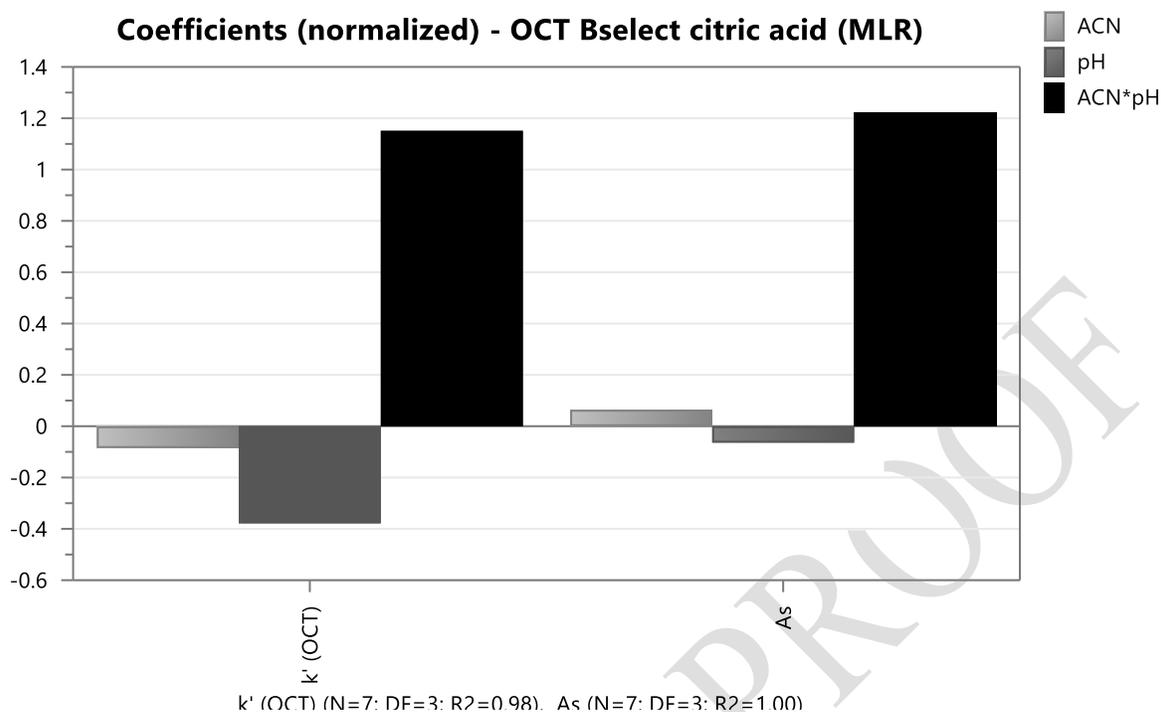


Fig..1 Normalized coefficients of the 2^2 Full Factorial DoE showing the effects of different chromatographic conditions: content of acetonitrile in mobile phase (ACN), pH value of buffer composed of 0.1M Citric acid/0.2M Na_2HPO_4 (pH) and their interaction (ACN*pH) on the chromatographic responses (retention factor (k') and Asymmetry factor (As) of octenidine).

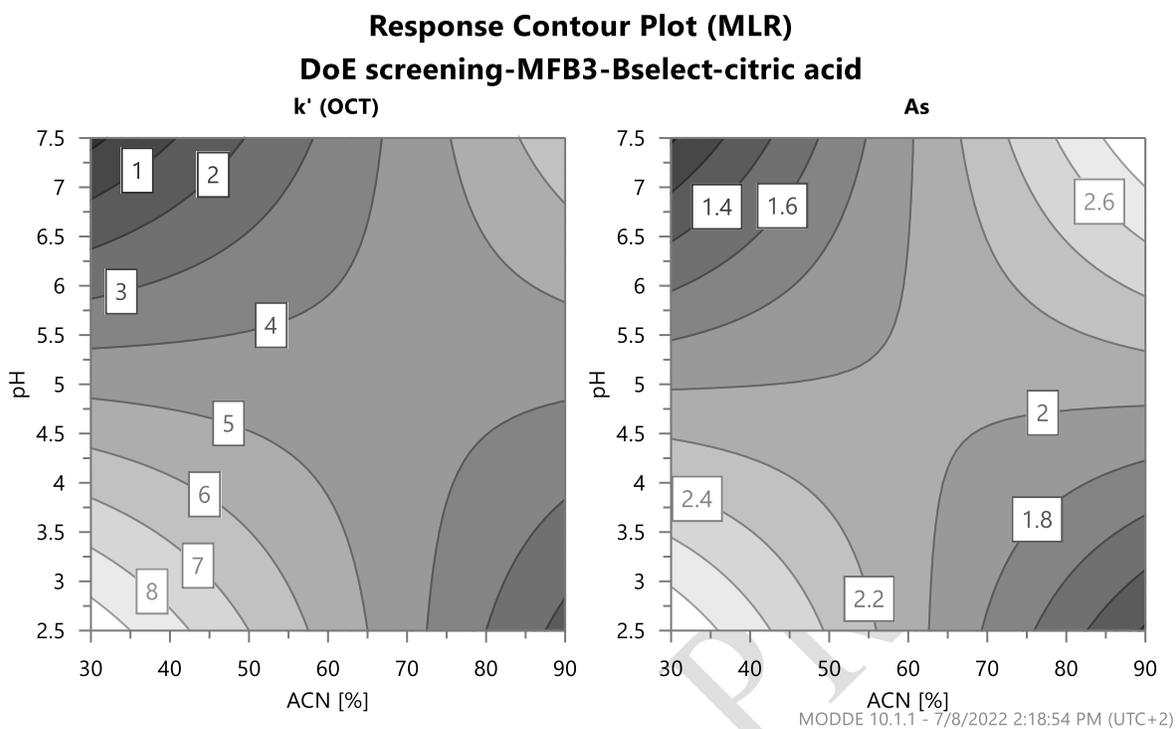


Fig. 2. response surface of the: influence of the acetonitrile content and pH value of the buffer on the retention factor (k') and asymmetry factor (As).

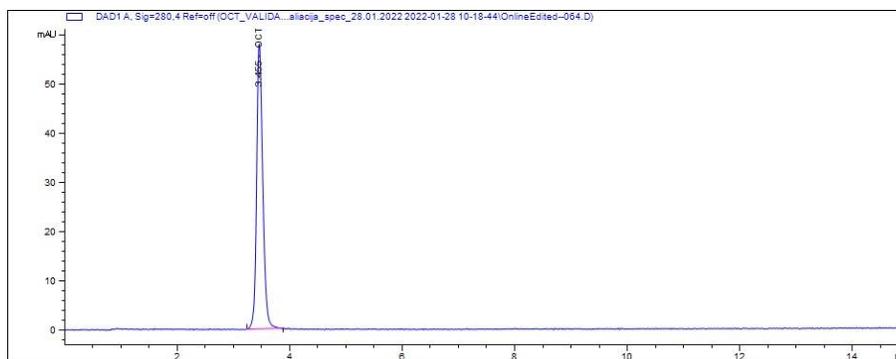


Fig. 3. Chromatogram of the standard solution of octenidine dihydrochloride