Short Communication

APPLICATION OF THE RESPONSE SURFACE METHODOLOGY FOR RP-HPLC ANALYSIS OF LIDOCAINE AND CETRIMONIUM BROMIDE

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Abstract

The successful analysis of drugs in complex pharmaceutical formulations by reversed–phase high performance liquid chromatography (RP–HPLC) relies upon the optimization of the chromatographic conditions, the sample preparation and post–column detection. HPLC separation of mixtures depends on a large number of factors. The optimization of RP–HPLC method defines the simultaneous influence of some important conditions, such as column type, mobile phase composition, pH of the mobile phase, column temperature and flow rate, to the separation and determination. In this paper the RP–HPLC method was applied for the optimal separation of lidocaine, cetrimonium bromide and color in the pharmaceutical pellets (Septalen® pellets). The selectivity factor values defined the optimal conditions, which were confirmed by analyzing the appropriate mathematical models. With the aid of the response surface methodology (RSM) it was possible to anticipate to a certain degree and precisely select optimal experimental conditions. Separations were performed on a Beckman Ultrasphere ODS 4.6 × 150 mm, 5 μm particle size column. Samples were introduced through a Rheodyne injector valve with a 20 μL sample loop. UV detection was performed at 208 nm. The optimization was performed within the pH range from 2.0 to 5.5; temperature range from 20 °C to 55 °C and composition of the mobile phase acetonitrile–water from (15:85 V/V) to (35:65 V/V). The three–D graphs, constructed with sixty-four experimental points, confirmed the optimal conditions for the separation of lidocaine, cetrimonium bromide and color in analyzed pharmaceutical preparations.

Key words: lidocaine, cetrimonium bromide, RP-HPLC, RSM, optimization

Introduction

The reversed–phase high–performance liquid chromatographic (RP–HPLC) method has been applied for the separation of lidocaine, cetrimonium bromide and color corrigent in some pellets. Lidocaine (2-(diethylamino)-*N*-(2,6-dimethylphenyl)-acetamide) is a local anesthetic drug with a pronounced antiarythmic and anticonvulsant effect. Cetrimonium bromide (*N*,*N*,*N*-trimethyl-1-hexadecaamminiumbromide) is an antiseptic drug characterized by the lack of a strong chromophore and, therefore, not

able to absorb at the higher wavelengths. The combination of cetrimonium bromide and lidocaine is used for pharyngeal irritation treatment. Pellet application needs the presence of color and taste corrigents.

In novel literature, for the determination of lidocaine in the mixture with cetrimonium bromide, the derivative spectrophotometric method was proposed.[1] The separation techniques, like thin-layer chromatography (TLC)[2,3] or high-performance liquid chromatography (HPLC)[4–6] were developed for the determination of lidocaine in different drug mixtures, biological fluids[7,8] or in the presence of its active metabolites in blood and other biological fluids,[9,10] as well as CE.[11] As an alternative to ion exchange chromatography, CE was used for the determination of cetrimonium bromide.[12,13]

The aim of these investigations was the optimization of RP–HPLC method which makes possible separation of basic drug – lidocaine; tetraalkylammonium compound – cetrimonium bromide; and color corrigent. The optimization enables the selection of optimal experimental conditions and provides maximum relevant information by analyzing experimental data. Optimization of the method was carried out applying the response surface methodology (RSM) for defining the optimal chromatographic conditions. RSM is a collection of mathematical and statistical techniques useful for analyzing problems where several independent variables influence a dependent variable or response, and the goal is to optimize this response.[14]

Results and discussion

The introduction of new HPLC method for routine quality control of pharmaceutical preparations is faced with series of preliminary investigations, which enables establishing the optimal experimental conditions and provide maximum relevant information by analyzing the experimental data. The optimization of the RP–HPLC method means to examine and to estimate the factors that affect investigated HPLC system. HPLC separation of mixtures depends on a large number of factors.[15] Among them there are the column type, the mobile phase composition, pH, column temperature and mobile phase flow rate.

A response surface method was carried out to obtain more information and to investigate the behavior of the response around the nominal values of the factors and to predict variation of the response inside or slightly outside the area investigated in

screening experiments.[16] Generally the large number of experiments are required by standard design applied in RSM, but in that way it is possible to anticipate to a certain degree and precisely select the optimal experimental conditions. RSM is a graph of system response (selectivity factor values) as a function of one or more factors, visual means of understanding how certain factors influence the system.

Investigated substances significantly differ in their physico-chemical properties. Lidocaine is a weak base (pK_a 7.86) and a very polar substance. Hydrogen bonding and ion exchange interactions can occur between the chromatographic support and the basic compound. These interactions produce peak tailing which affect resolution, sensitivity and reproducibility. Cetrimonium bromide as a tetraalkylammonium compound has a strong silanophilic action at high pH values and it was used as a mobile phase modifier.[17] Having on mind molecular characteristics of lidocaine and cetrimonium bromide, as well as presence of color with expressive adsorption, their separation must be carefully studied.

Applying optimization of RP–HPLC method for the separation of lidocaine, cetrimonium–bromide and color, the optimal chromatographic conditions, such as composition of the mobile phase, pH of the mobile phase and column temperature, were established. The selectivity factor value was the response, which was followed to define the optimal conditions.

Influence of the mobile phase composition on the separation of lidocaine, cetrimonium bromide and color was investigated following retention times, peak symmetry and behavior of the color. It was important to obtain the most appropriate conditions when the best retention, resolution and acceptable peak symmetry of investigated substances could be achieved.

It is well known that the degree of ionization of solutes, stationary phase and mobile phase additives are affected by the pH and may lead to better selectivity. In order to overcome the interactions of lidocaine and cetrimonium bromide with the chromatographic support it was necessary to work at low pH values when silanol groups were mostly uncharged.

In order to set the best possible retention and selectivity simultaneous variation of pH of the mobile phase and aqueous/organic ratio is recommended. The ranges of investigated factors were defined and the study was done on related influence of:

1) percentage of acetonitrile (%ACN) content and pH of the mobile phase and 2) percentage of acetonitrile (%ACN) content and column temperature. For each of them 64 experiments were performed. Based on the performed experiments, coefficients were calculated characterizing the polynomes of second order and three-D graphs were constructed as well.

For the % of acetinitrile/pH of the mobile phase system the following equation was obtained:

$$z = 1.048 - 0.021x + 0.042y + 0.001x^2 - 0.002xy + 0.001y^2 \qquad \text{(Eq. 1)}$$

where is: z-selectivity factor for cetrimonium bromide and color separation, x- % of acetonitrile and y- pH of the mobile phase.

Separation of the color and lidocaine, for the % of acetinitrile/pH of the mobile phase system, was defined with the equation:

$$z = - 6.413 + 0.091x + 6.477y + 0.001x^2 - 0.134xy - 0.084y^2 \qquad \text{(Eq. 2)}$$

where is: z-selectivity factor for color and lidocaine separation, x- % of acetonitrile and y- pH of the mobile phase.

Three-D graphs are presented in Figure 1A and 1B.

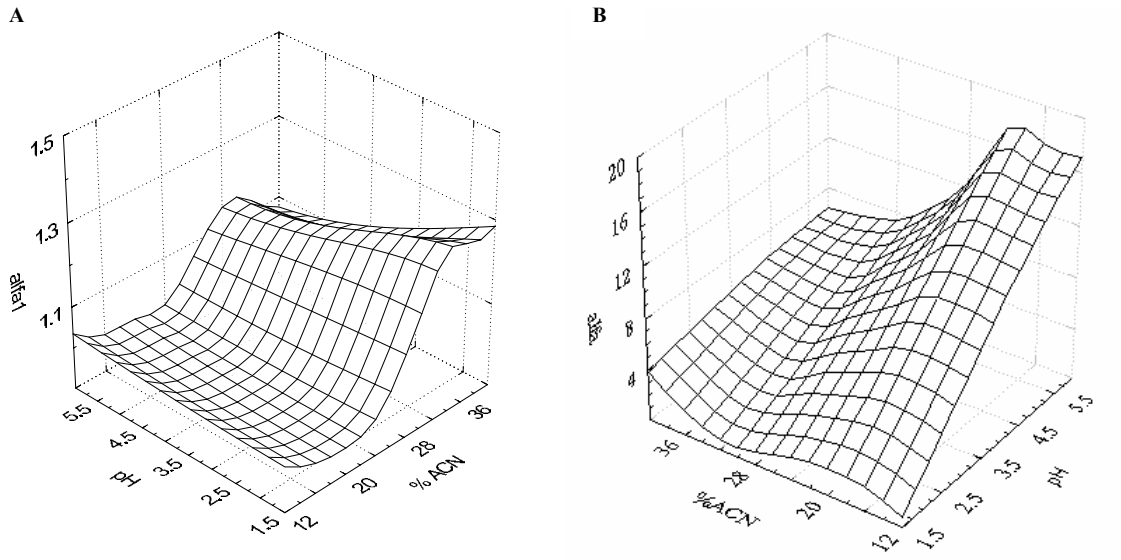

Figure 1. A. Three–D graph αC/CB = f (%ACN, pH); B. Three–D graph αL/C = f (%ACN, pH) (αC/CB – selectivity factor of color and cetrimonium bromide) (αL/C – selectivity factor of lidocaine and color).

For the % of acetinitrile/column temperature of the mobile phase system the following equation was obtained:

$$z = 1.022 - 0.008x + 0.001y + 0x^2 + 1.531e\text{-}5xy - 1.69e\text{-}5y^2 \quad \text{(Eq. 3)}$$

where is: z-selectivity factor for cetrimonium bromide and color separation, x- % of acetonitrile and y- column temperature.

Influence of the % of acetinitrile/column temperature system on the color/lidocaine separation was given with equation:

$$z = 11.996 - 0.006x - 0.026y - 0.004x^2 - 6.047e\text{-}5xy + 0y^2 \quad \text{(Eq. 4)}$$

where is: z-selectivity factor for cetrimonium bromide and lidocaine separation, x- % of acetonitrile and y- column temperature.

Three-D graphs are presented in Figure 2A and 2B.

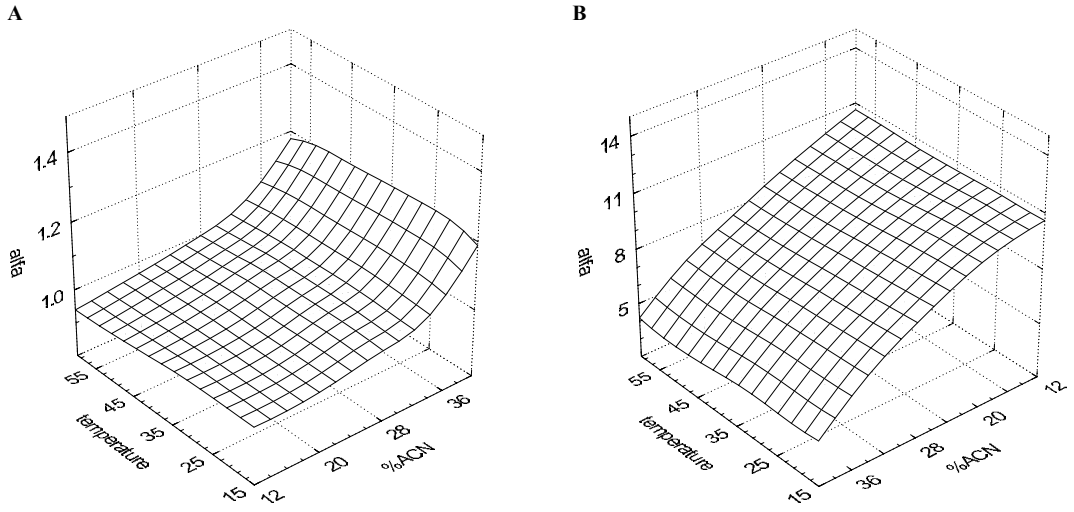

Figure 2. A. Three–D graph $\alpha_{C/CB}$ = f (%ACN, t °C) B. Three–D graph $\alpha_{L/C}$ = f (%ACN, t °C) ($\alpha_{C/CB}$ – selectivity factor of color and cetrimonium bromide) ($\alpha_{L/C}$ – selectivity factor of lidocaine and color)

Analysis of three–D graphs showed that the optimal composition of the mobile phase should be established according to the separation of cetrimonium bromide and color. The best separation of these two adjacent peaks was achieved with the mobile phase acetonitrile–water (28:72 V/V) which is manifested with expressive "reef" on the three–D graph – Figure 1A. With pH values of the mobile phase from 2.0 to 5.5 and acetonitrile–water (28:72 V/V) mobile phase a good separation could be achieved.

In order to set the best possible lidocaine peak symmetry variation of pH of water phase, e.g. pH of the mobile phase had to be performed. Due to the reduction of ionization of acidic –SiOH sites by employing mobile phases of low pH (pH < 4.0) better lidocaine peak symmetry was obtained. Working at a low pH value (pH 2) lead to a complete ionization of lidocaine so the retention was somewhat affected, but the peaks were sharp and narrow.

Three–D graphs $\alpha_{C/CB}$ = f (%ACN, t °C) –Figure 2A, and $\alpha_{L/C}$ = f (%ACN, t °C) Figure 2B, show that a column temperature have no important influence on the separation. Representative chromatogram of laboratory mixture is given in Figure 3.

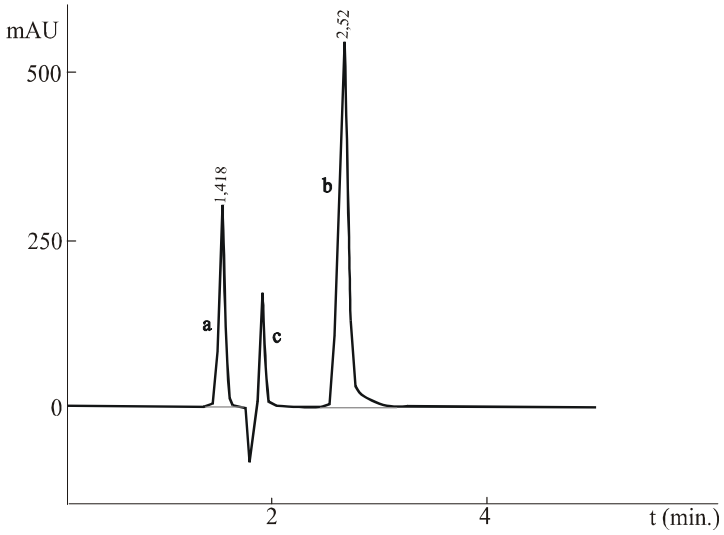

Figure 3. Representative chromatogram of laboratory mixture of cetrimonium bromide (a), lidocaine (b), color (c)

Conclusions

The RP–HPLC method for the separation and analysis of lidocaine, cetrimonium bromide and color corrigent was optimized applying response surface methodology. The large number of experiments, required by standard design applied in RSM, enabled estimation of factors that affect investigated HPLC system and precise selection of the optimal experimental conditions. Such procedure provided some valuable informations concerning chromatographic behavior of investigated substances. Optimized method can be used for separation, identification and simultaneous determination of lidocaine and cetrimonium bromide in pharmaceutical dosage forms.

Experimental

Chemicals

All the chemicals and the reagents were of an analytical reagent grade and water was re-distillated and filtered through a membrane filter. Acetonitrile – gradient grade (*Merck*, Darmstadt, Germany), sodium hydroxide (*Zorka Pharma*, Šabac, SCG) and 85% ortophosphoric acid (*Carlo Erba*, Italy) were used to prepare the mobile phases. The USP standard substances of lidocaine and cetrimonium bromide, as well as the working standard of color, were obtained from *Krka*, Novo Mesto, Slovenia.

Chromatographic conditions

The chromatographic system Hewlett Packard 1100 consisted of HP 1100 pump, HP 1100 UV–VIS Detector and HP ChemStation integrator. Separations were performed on a Beckman Ultrasphere ODS 4.6 mm × 15 cm, 5μm particle column. The samples were introduced through a Rheodyne injector valve with a 20 μL sample loop. The flow rate of the mobile phases was 1 mL/min. UV detection was performed at 208 nm.

For the optimization of the separation the following mobile phases, consisting of acetonitrile–water mixture, were prepared: 15:85 V/V; 20:80 V/V; 23:77 V/V; 25:75 V/V; 28:72 V/V; 30:70 V/V; 33:67 V/V and 35:65 V/V. pH of the mobile phases was adjusted with 85% ortophosphoric acid at 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5. Column temperature varied from 20 °C to 55 °C, while the pH of the mobile phases was adjusted precisely at 2.0.

Optimal conditions for the separation of lidocaine, cetrimonium bromide and color, with the good resolution and peak symmetry, were obtained using Beckman Ultrasphere ODS 150 x 4.6 mm, particle size 5 μm column. Mobile phase consisted of acetonitrile and water. Water phase was prepared by mixing 835 mL of redestillated water and 65 mL of 1.0 $molL^{-1}$ sodium hydroxide solution. pH of the water phase was adjusted to 2.0 with 85% ortophosphoric acid and than the sufficient amount of redestillated water was added up to 1000 mL. The composition of the mobile phase was acetonitrile–water (28:72 V/V).

Laboratory mixture

Laboratory mixture, which corresponded to analyzed pellets, was prepared of lidocaine (0.1 mg/mL), cetrimonium bromide (0.2 mg/mL) and appropriate concentration of color in acetonitrile: water (28:72 V/V) mixture.

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Povzetek

Za uspešno analizo učinkovin v farmacevtskih pripravkih z reverznofazno tekočinsko kromatografijo (RP-HPLC) so potrebni pravi kromatografski pogoji, priprava vzorca in detekcija. Analiza zmesi z uporabo HPLC je odvisna od množice dejavnikov, kot so tip kolone, pretok, sestava in pH mobilne faze ter temperatura kolone. V tem prispevku opisujemo separacijo lidokaina, cetrimonijevega bromida in barvila v farmacevtskem pripravku (Septalen® tablete). Ločevanje smo izvedli z uporabo kolone Beckman Ultrasphere ODS 4,6×150 mm z velikostjo delcev 5 μm, vzorec pa smo v sistem vnesli z uporabo injektorja Rheodyne z zanko prostornine 20 μL. Spojine smo določili z UV detekcijo pri 208 nm. Optimizacijo pH v območju 2,0-5,5, temperature v območju 20-55 °C in sestave mobilne faze acetonitril-voda od 15:85 V/V do 35:65 V/V smo izvedli s pomočjo 3D diagramov iz po 64 eksperimentalno določenih točk. S parametrom selektivnosti in z uporabo metode odzivnih površin smo lahko natančno izbrali prave pogoje in jih deloma napovedali.