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Statistical Optimization Applied to Simultaneous Determination of Maprotiline, Desipramine, and Moclobemide by Capillary Zone Electrophoresis

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Summary. A method of Capillary Zone Electrophoresis (CZE) for separation of three most-frequently prescribed antidepressants in the market: maprotiline, desipramine, and moclobemide was developed. The proposed method is fully validated for a ternary laboratory mixture but it is also suitable for individual determination of the investigated components in pharmaceutical dosage forms.

Since the preliminary investigations did not show complete separation because of close migration time of desipramine and maprotiline, a complete set, 2^3 experimental design was applied. All the factors that affect separation as well as their mutual interactions were investigated. Voltage, temperature of capillary and the *pH* of phosphate buffer were independent variables or factors to be investigated in two levels. Applying response surface methodology, from experimental points the appropriate graphs were constructed and optimal chromatographic conditions for the separation were defined. The optimum conditions were: running voltage of 20 kV, phosphate buffer ($pH = 2.35$), temperature 25°C, and UV detection at 200 nm. An uncoated (fused silica) capillary (50 μm i.d.) with a total length of 50 cm and a distance of 47 cm between the injection and detection points was used.

Keywords. Experimental design; Capillary zone electrophoresis; Maprotiline; Desipramine, Moclobemide.

Introduction

Up to now the most common method for optimizing the experimental conditions is considered as *one factor at a time*. Such an approach does not allow selecting variables that have the most influence on the response and does not take into account the possible interdependence between variables. On the other hand, a multivariate method allows simultaneous changes of all chosen factors affecting response and avoids useless and time-consuming experiments [1]. For optimizing conditions of the experiments two statistical models are usually used: factorial design and response surface diagram. The goal of factorial design is to identify the critical factor in the analytical procedure (critical factors are quantitative and qualitative factors which are believed to affect the results). The goal of applying response surface design is to determine the specification limits and predict the variation of the response (the response surface plot allows visualization of the variations of the response as a function of the level of the factors). A literature search showed that experimental design has been applied for spectrophotometric [2] and adsorptive stripping voltammetric determination [3], as well as for validation in HPLC method [4, 5] for robustness testing in HPLC [6, 7] and CZE method [8].

In this paper, full factorial design has been applied for optimizing CZE conditions for the simultaneous determination of maprotiline (*N*-methyl-9,10-ethanoantracene-9(10*H*)-propanamine, hydrochloride), desipramine (10,11-dihydro-*N*-methyl-5*H*-dibenz-[*b*,*f*]azepine-5-propamine, hydrochloride), and moclobemide (4-chloro-*N*-[2-(4-morpholinyl)ethyl]-

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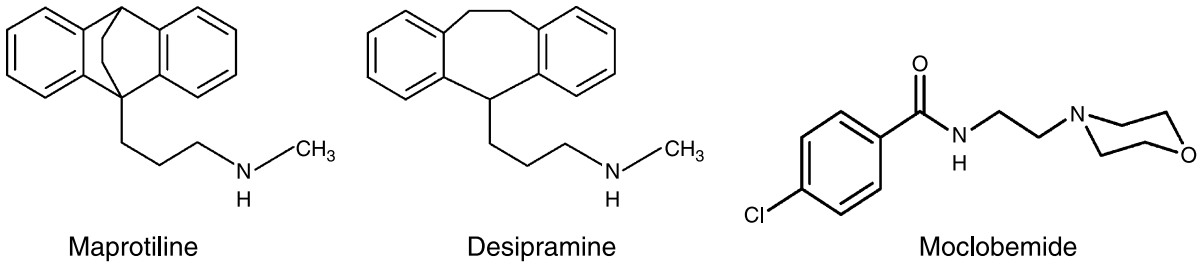

Fig. 1. Chemical structure of desipramine, maprotiline, and moclobemide

benzamide) in a mixture. Moclobemide (reversible inhibitor of MAO-A), maprotiline (sometimes described as tetracyclic, rather than tricyclic antidepressant), and desipramine (may be considered the parent compound of TCAs) are widely used as antidepressants in clinical practice [9, 10]. The structures of these compounds are shown in Fig. 1.

Although various methods have been proposed for qualitative and quantitative determination of a variety of antidepressants, including maprotiline, desipramine, moclobemide, and their metabolites [11–22], searching literature showed no data related to simultaneous CZE determination of the mentioned antidepressants. Thus, the primary objective was to establish the optimal conditions for the CZE separation of compounds belonging to the same pharmacological group and having similar structure (maprotiline and desipramine).

Results and Discussion

During preliminary investigations the electrophoretic behavior of antidepressants was investigated using different buffers, applied voltage, and temperature of capillary. The most acceptable peak shapes were achieved with phosphate buffer at low *pH*. Phosphate buffer is very effective because of its high buffering capacity and low UV absorbance. At low *pH*, the electroendoosmotic flow (*EOF*) is reduced, but in acidic medium all examined substances, possessing secondary amino groups, will be protonated. Moreover, maprotiline and desipramine having similar structures and thus similar physical-chemical properties show close migration times. Thus, separation under preliminary conditions was not achieved and required further optimization.

For defining optimal conditions the full factorial design, for a total of 8 experiments, was performed. In full factorial design the number of experiments corresponds to all possible combinations of selected factors and levels [1] meaning that for great number of factors, for example 8, at two levels, 256 experiments are required. Full factorial design is most useful when the number of factors is relatively limited. That is the reason why ''only'' three factors were considered as possibly influencing the outcome of the experiments. Typical factors in CZE are, for example, the *pH*, nature of electrolyte or electrolyte concentration, the applied voltage, and temperature. The primary goal was to achieve acceptable electrophoretic separation since maprotiline and desipramine have close migration time.

Electrophoretic migration of ions follows the equation:

$$\mu_{EM} = (l_{ef}/t_M) \cdot (l_{tot}/V)$$

where μ_{EM} is electrophoretic migration of ions, l_{ef} effective length of capillary, t_M migration time, l_{tot} total length of capillary and ΔV is applied voltage. At constant values of l_{ef} and l_{tot} electrophoretic migration depends on migration time and applied voltage. Thus, these two values were chosen as system response and variable, respectively. To sum up, three variables were considered as possibly influencing the outcome: the applied voltage (*A*), the temperature of capillary (*B*) and the *pH* of phosphate buffer (*C*) with migration time as system response.

In order to maximize the information that could be obtained from experimental data different levels of these variables were selected. The design matrix of the eight treatment combinations of low (−) and high (+) levels of factors as well as outcomes of experiments is shown in Table 1.

By applying analysis of variance, the statistical significance of each effect was tested by comparing the mean square against an estimate of the experimental error. The statistical test showed that

Table 1. Scheme of the experiment

Trial	Factor level*			Response (t_M/min)		
	Applied voltage	Temp. of capillary	*pH* of buffer	Maprotiline	Desipramine	Moclobemide
1	−	−	−	11.5	13.96	10.7
2	+	−	−	14.3	15.2	14.04
3	+	+	−	14.23	12.68	14.52
4	−	+	−	13.26	13.95	12.73
5	+	+	+	11.82	12.6	10.92
6	−	+	+	10.46	12.25	9.5
7	−	−	+	9.76	8.6	8.8
8	+	−	+	11.37	11.69	10.59

* Low (−) and high (+) levels of the following factors:

Factor	(−)	(+)
A: applied voltage	15 kV	20 kV
B: temp. of capillary	15°C	25°C
C: *pH* of buffer	2.35	2.98

Table 2. Estimates of factor effects

	Maprotiline		Desipramine		Moclobemide	
	Factor effects	*p*	Factor effects	*p*	Factor effects	*p*
Intercept	12.09		12.62		11.48	
A	0.84	**0.0207**	0.43	0.2807	1.04	**0.0140**
B	0.36	0.102	0.25	0.4753	0.44	0.0710
C	−1.23	**0.0098**	−1.33	**0.0446**	−1.52	**0.0066**
AB	−0.26	0.1689	−0.66	0.1529	−0.24	0.1940
AC	−0.1	0.5016	0.43	0.2747	−0.24	0.1940
BC	−0.067	0.6382	0.89	0.0931	−0.19	0.2759
ABC	0.20	0.2496	−0.029	0.9303	0.15	0.3582

two effects had *p*-values (*probability*) less than 0.05 indicating that they were significantly different from zero at 95% confidence level (bolded values in Table 2). The significant factors were found to be applied voltage (*A*) and *pH* of buffer (*C*) for maprotiline and moclobemide and *pH* of buffer (*C*) for desipramine. Temperature of capillary has no statistical significant influence on migration time of investigated compounds and in following experiments was kept constant at 25°C. Mutual interactions of these factors were not of great importance ($p < 0.05$) as well.

In order to identify the optimum experimental conditions a response surface diagram was developed. A response surface can simultaneously represent two independent and one dependent variable when a mathematical relationship between variables can be assumed.

The study was done on related influence of applied voltage and *pH* of buffer to migration time of examined substances. For each of them 20 experiments were performed. Lack of fit (LOF) test was carried out in order to evaluate which one of suggested models is appropriate for a given response. LOF indicates the variation of the data around the fitted model. If a model shows lack of fit, it should not be used to predict the response – a small *F* value and high *p* value, greater than 0.1, are good in test (Table 3).

Based on the obtained results, coefficients were calculated characterizing the polynomes of second order and two factorial interaction. For the applied

Table 3. Lack of fit tests

Source	Sum of squares	*DF**	Mean square	*F* value	*p*	
Maprotiline						
Linear	51.01	6	8.50	3.87	0.1054	
2FI**	51.01	5	10.20	4.65	0.0809	
Quadratic	*23.96*	*3*	*7.99*	*3.64*	*0.1221*	*Suggested*
Cubic	20.94	1	20.94	9.54	0.0366	
Desipramine						
Linear	*12.21*	*6*	*2.04*	*0.37*	*0.8690*	*Suggested*
2FI	*5.24*	*5*	*1.05*	*0.19*	*0.9519*	*Suggested*
Quadratic	5.18	3	1.73	0.31	0.8177	
Cubic	0.77	1	0.77	0.14	0.7292	
Moclobemide						
Linear	30.38	6	5.06	48.63	0.0011	
2FI	30.01	5	6.00	57.63	0.0008	
Quadratic	*0.68*	*3*	*0.23*	*2.17*	*0.2343*	*Suggested*
Cubic	1.12	1	0.12	1.15	0.3441	

* *DF* Degrees of freedom
** *2FI* Two factorial interaction

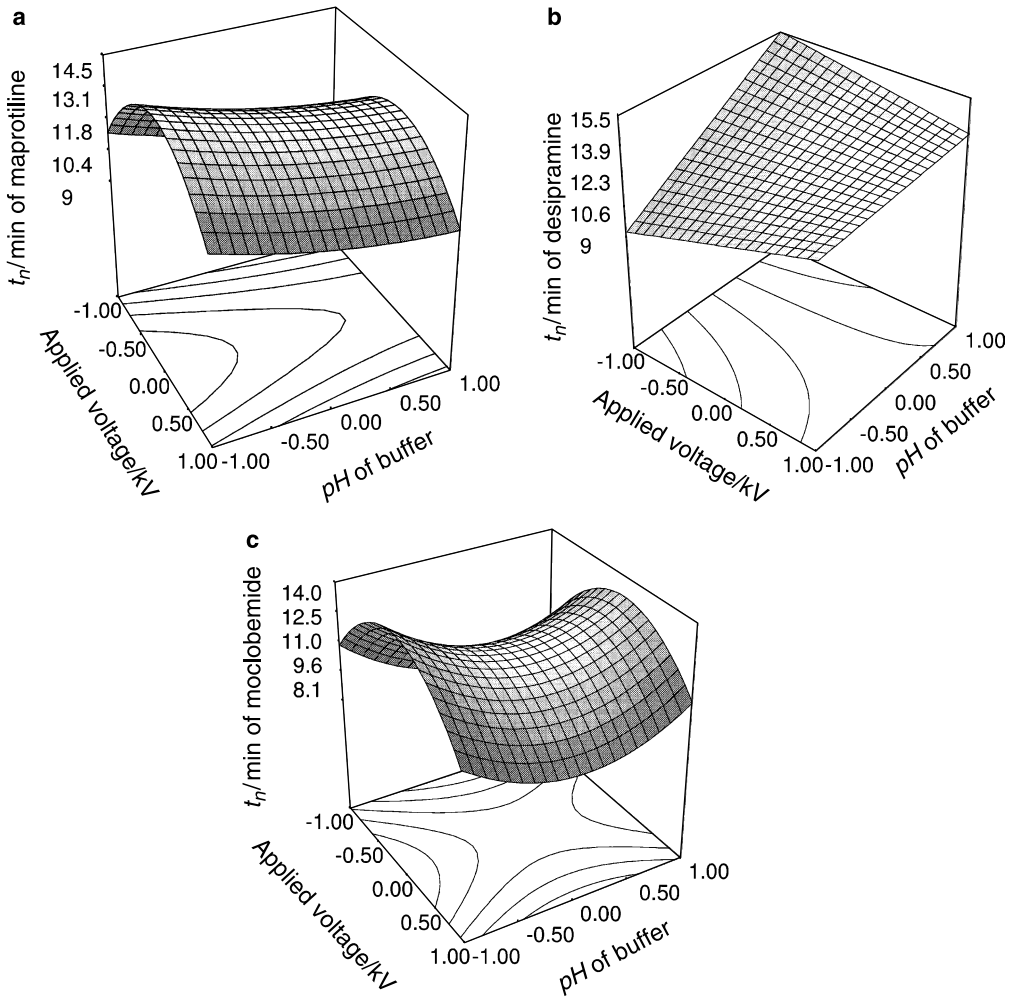

Fig. 2. Three-dimensional graph of $t_M = f$ (applied voltage and *pH*) for maprotiline (a), desipramine (b), and moclobemide (c)

voltage/pH buffer system the equations of t_M were obtained for maprotiline, desipramine, and moclobemide:

$$t_{M\,map} = 13.12 + 0.28A - 0.97C - 3.01A^2 + 0.35C^2 + 0.03AC \quad (1)$$

$$t_{M\,des} = 12.77 + 0.36A + 1.78C - 1.32AC \quad (2)$$

$$t_{M\,moc} = 11.57 + 0.16A - 0.5C - 3.18A^2 + 1.88C^2 + 0.31AC \quad (3)$$

where A is the applied voltage and C is pH of buffer. For desipramine, based on statistical parameters both linear and two factorial interaction model of response surface were suggested, but $2FI$ model, related to less value of F, was chosen.

Three dimensional graphs in coded factor space are presented in Fig. 2.

Analyzing the three-dimensional graph, a difference between the obtained graphs for maprotiline and desipramine is evident. The negative estimates in the fitted model (Eq. (1)) cause the surface to fold downward quadratically in factor A and less rapidly in factor C. By careful inspection of Fig. 2a it was noticed that the "ridge" of the surface is tilted with respect to the factor axes from the middle toward the front and back. The fitted model (Eq. (2)) gives esti-

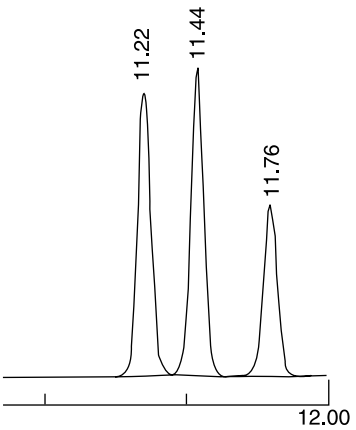

Fig. 3. The electrophorogram of the mixture of moclobemide ($t_M = 11.22$), maprotiline ($t_M = 11.44$), and desipramine ($t_M = 11.76$); phosphate buffer ($pH = 2.35$), applied voltage 20 kV, temperature 25°C

Table 4. Statistical parameters for individual calibration curves

	$y = a + bx$	r	LOD^*	LOQ^*	$t_a{}^{**}$
			$\mu g\,cm^{-3}$	$\mu g\,cm^{-3}$	
Maprotiline	$y = 816.2 + 5875x$	0.9990	0.38	1.28	1.085
Desipramine	$y = 1360.1 + 4720.5x$	0.9993	0.4	1.32	1.786
Moclobemide	$y = 1780.1 + 5604.2x$	0.9987	0.53	1.78	2.19

* The limit of detection (LOD) and limit of quantization (LOQ) were calculated as $LOD\ 3\sigma/S$ and $LOQ\ 10\sigma/S$, where σ is the standard deviation and S the intercept determined from the corresponding calibration curve
** $t_a = 2.365$ ($p = 0.05$, $f = 7$)

Table 5. Statistical analysis of the results obtained by determination of maprotiline, desipramine and moclobemide in the bulk drugs and pharmaceutical dosage forms

Sample	Concentration	Found	Sd	RSD	Recovery
	$\mu g\,cm^{-3}$	$\mu g\,cm^{-3}$	μg	%	%
Maprotiline	1	1.029	1.26	1.53	98.96–100.95
bulk drug	10	10.09	1.24	0.12	99.91–101.20
	15	15.45	1.58	0.12	99.29–101.74
Desipramine	1	1.034	1.36	1.32	99.79–101.73
bulk drug	10	10.49	1.27	2.14	99.58–101.07
	15	15.28	1.13	1.80	98.76–102.73
Moclobemide	1	1.011	1.23	1.46	98.79–100.61
bulk drug	10	10.29	1.14	0.59	99.64–101.36
	15	15.67	0.76	0.71	99.55–101.36
Maprotilin® *tablets*	5	5.04	1.62	1.25	98.83–102.47
Pertofran® *dragee*	2.5	2.61	1.78	2.08	97.86–102.94
Auromid® *film tablets*	6	6.12	1.24	0.78	99.28–102.74

mates of a first-order effect of the factor *A*, a first-order effect of the factor *C*, and a second-order interaction effect between two factors. From the graphs it was concluded that increasing of applied voltage results in increasing of migration time of desipramine and decreasing of migration time of maprotiline. Moreover, the three-dimensional graph obtained for maprotiline shows acceptable results (low value of migration time) at all four corners of the diagram. Considering the fact that phosphate buffer is very effective at low *pH* and contributes to reducing of *EOF*, buffer of $pH = 2.35$ was chosen. Under this *pH*, the best separation was achieved by applying high voltage (20 kV).

A representative electrophoregram of the mixture obtained under selected conditions is given in Fig. 3.

The method was validated. Interfering peaks were not detected, indicating good selectivity of the applied extraction procedure. Linear dependence of the peak area *versus* concentration was determined in the concentration range 0.6–20 $\mu g\,cm^{-3}$ for maprotiline, 1–20 $\mu g\,mm^{-3}$ for desipramine, and 1–20 $\mu g\,mm^{-3}$ for moclobemide. Parameters of the linear regression equation were calculated for each component (Table 4). Precision of the procedure was assessed by analyzing ten solutions containing known quantities of the investigated compounds. Low values of relative standard deviation (*RSD*) for repeatability, $RSD < 2.5\%$, and high recovery (Table 5) indicate very good precision of the proposed method.

Conclusion

A method for separation of three antidepressants: maprotiline, desipramine, and moclobemide by CZE was developed using experimental design. These results give a demonstration of superiority of the multivariate approach over the one-variable-at-a-time method allowing important factors to be considered simultaneously and avoiding useless and time-consuming experiments. Considering the high rate of abuse of the above mentioned antidepressants and the notion that they are occasionally used at the same time, methods for their simultaneous determination may be relevant for therapeutic drug monitoring in clinical toxicology. The developed CZE method is rapid, each determination takes approximately 15 min including the washing procedure, and can be applied in routine control of pharmaceutical preparations.

Materials and Methods

Reagents and Standards

Methanol and hydrochloric acid were obtained from Zorka (Sabac, Serbia), and 85% phosphoric acid was supplied by Carlo Erba (Milan, Italy). Water was of Mili-Q quality from Milipore Corporation, USA. Analytical grade sodium dihydrogen phosphate was purchased from Merck, Alkaloid (Skopje, Macedonia). Sodium hydroxide was supplied by Euro Hemija (Belgrade, Serbia). Working standards of maprotiline-hydrochloride were obtained from Zdravlje (Leskovac, Serbia), while standards of desipramine-hydrochloride and moclobemide were obtained from Sigma-Aldrich.

Apparatus

CZE separation was performed with a fully automated Bio Focus 3000 Capillary Electrophoresis system (Bio Rad Laboratories) equipped with an on column, high speed scanning UV-VIS detector with detection at 200 nm. An uncoated (fused silica) capillary (50 μm i.d.) with a total length of 50 cm and a distance of 47 cm between the injection and detection points was used. Before using, the capillary was treated successively for 30 s with 0.1 *M* HCl, water, 0.1 *M* NaOH, and running buffer. For electrophoretic runs, a phosphate buffer ($pH = 2.35$) was used.

Multivariate regression calculations were supported by the statistical graphic software system "Design-Expert" ver. 6.0.

Standard Mixtures

A mixture of stock standard solutions was prepared by dissolving the respective working standard compounds in a mixture of phosphate buffer $pH = 2.35$ and methanol, 1:1 volumetric ratio (methanol was added to improve the solubility) to obtain the concentration of 2 $mg\,cm^{-3}$ of each of the examined substances. Working standard solutions were prepared by diluting stock solutions to give the concentrations of 0.02 $mg\,cm^{-3}$ of each of the examined substances. Series of calibration standards were prepared by diluting the standard stock solutions in volumetric ratios 1:1, 1:2, 1:3, 1:4, 1:5, 1:8, 1:10, and 1:20. The ternary mixture was prepared by mixing appropriate volumes of the standard.

Preparation of Sample Solutions

Twenty tablets of maprotiline-hydrochloride or moclobemide (Maprotilin® tablet contains 25 mg of maprotiline-hydrochloride or Auromid® film tablet contains 150 mg of moclobemide) were randomly selected, accurately weighed and the average value was calculated. The tablets were crushed and accurately weighed portions containing equivalents of 25 mg maprotiline-hydrochloride or 150 mg moclobemide were transferred into 25 cm^3 volumetric flasks, dissolved in 15 cm^3 mixture of phosphate buffer $pH = 2.35$ and metanol (1/1 *v*/*v*), and sonicated for 15 min at room temperature. After sonication, the flasks were filled up with same solvent to the mark and the obtained solutions were filtered through 0.2 μm Millipore filters. 5 cm^3 of solution of maprotiline-hydrochloride and 1 cm^3 of solution of moclobemide thus prepared were diluted with sol-

vent to a total volume of 100 cm^3. 1 cm^3 of those obtained solutions were diluted again with solvent to a total volume of 10 cm^3. Concentrations of solutions were 0.005 mg cm^{-3} of maprotiline-hydrochloride and 0.006 mg cm^{-3} of moclobemide.

Pertofran® dragee solution was prepared by transferring the equivalent of one dragee (containing 25 mg of desipramine-hydrochloride) into a 100 cm^3 volumetric flask, dissolving in 30 cm^3 mixture of phosphate buffer *pH* 2.35 and metanol (1/1 *v*/*v*). After sonication, the flask was filled up with solvent to the mark and the solution filtered through a 0.2 μm Millipore filter. 1 cm^3 of the filtrate was diluted to 100 cm^3 with ethanol, yielding a solution of desipramine of the concentration 0.0025 mg cm^{-3}.

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