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Selection of background electrolyte for CZE analysis by a chemometric approach Part II. Separation of a mixture of basic beta-blocker drugs

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Abstract

In the first part of this study a chemometric approach to choose a suitable background electrolyte for CZE analysis was introduced. Two hundred and twenty-two possible electrolytes were previously characterized by means of the descriptors pH, conductivity, ionic strength and relative viscosity and the approach was applied to the separation of a mixture of acidic drugs. In this second part, another application concerning the analysis of basic drugs is presented. The test mixture was made of eight beta-blocker drugs. According to the basic nature of the analytes, the original data set was reduced to a new subset of 117 objects with pH less than or equal to 7, and after computing principal components the new set of objects was represented in a two-dimensional space. Ten objects to be tested in CZE, capable of covering homogeneously the principal component space, were selected by means of Kennard–Stone algorithm. The data set was further reduced around the BGEs which gave the best results, and a new set of electrolytes to be tested was selected. Using pH 4 citrate buffer, an electropherogram with baseline resolution was obtained in 10 min. A Doehlert design was run to further reduce analysis time, and applying the optimized conditions (voltage, 23 kV; temperature, 26 °C) the separation was obtained in about 7 min.

Keywords: Background electrolyte selection; Beta-blocker drugs; CZE; Experimental design; Principal component analysis

1. Introduction

In capillary electrophoresis the composition of the background electrolyte (BGE) is fundamental in order to modify the analyte mobility and the electroosmotic flow (EOF) and thus to reach a good quality separation in terms of efficiency, resolution and analysis time. In Part I of this study [1], a chemometric approach for the selection of a good background electrolyte (BGE) for CZE analysis of small drug molecules was discussed.

The approach is based on principal component analysis [2–4] and experimental design [2,5]. Briefly, the proposed strategy involved the following steps: (i) characterization of a large set of possible BGEs by appropriate physico-chemical descriptors; (ii) reduction of the original data set considering the characteristics of the specific CZE analysis; (iii) visualization of the new data set by means of principal component analysis; (iv) selection of a limited number of BGEs to be tested in CZE capable of covering principal component space; (v) choice of a suitable BGE; (vi) response surface methodology for finding the global optimum conditions.

In particular, in the initial part of the work a characterization of 222 possible BGEs by the descriptors pH [6–10], conductivity [11–14], ionic strength [6,8,11,14–17] and relative viscosity [6,8,11,15,16] was made and the approach was applied to the separation of a mixture of six acidic arylpropionic anti-inflammatory drugs. The procedure led to the complete resolution of the analytes, obtained in less than 10 min.

Hence, this part of the work presents an application in the field of basic drugs, in order to determine the real usefulness and suitability of the proposed approach. The application concerns the separation of eight beta-blocker drugs.

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2. Materials and methods

2.1. Chemicals

All chemicals and reagents used were of analytical-reagent grade with no further purification. All the beta-blocker drugs (acebutolol hydrochloride (ACE), alprenolol hydrochloride (ALP), atenolol (ATE), labetalol hydrochloride (LAB), metoprolol sodium tartrate (MET), nadolol (NAD), pindolol (PIN), propranolol hydrochloride (PRO)) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All the substances used to prepare background electrolytes (see the first part of the study [1] for a complete list) were from Sigma–Aldrich. Ultrapure water was used throughout the study and was obtained with a Milli-Q system (Millipore/Waters, Milford, MA, USA).

2.2. Solutions

Standard stock solutions of beta-blocker drugs were prepared in water, apart from atenolol, nadololol and pindolol. These latter drugs were dissolved in acidic conditions (2.5 mL of 0.1 M acetic acid) and the solution obtained was then diluted with water up to 50 mL to obtain a final concentration of about 5.0×10^{-4} M of the drugs. Working standard solutions were prepared by diluting 1:10 with water to obtain a final concentration of 5.0×10^{-5} M for all the analytes. All the standard stock solutions were stored at 4 °C and used within 1 week, while all the working standard solutions were prepared daily.

Compositions of all considered BGEs (pH range 2–12) are reported in the first part of this study [1]. The standard run buffer (I) employed for the analysis of beta-blockers was prepared adding 50 mL of 0.2N citric acid to 20 mL of 0.2 M NaOH.

2.3. Capillary electrophoretic conditions

The general capillary electrophoretic conditions (instrumentation, capillaries, capillary washing, injection) were previously described by the authors in the first part of the study [1]. Detection wavelength was 195 nm. Initial screening of the electrolytes was carried out setting capillary temperature at 25 °C and voltage at 18 kV.

The final optimized conditions were: standard run buffer (I), voltage 23 kV and temperature 26 °C (generated current about 45 μA).

2.4. Calculations and software

Resolution values R were calculated according to the European Pharmacopoeia [18].

Principal component analysis was carried out by means of PARVUS software package [19], while NEMROD-W software package [20] was employed to select BGEs by means of Kennard–Stone algorithm [21] to generate experimental designs and to perform statistical analysis of the data.

3. Results and discussion

The considered application concerned the separation of eight beta-blocker drugs: acebutolol, pK_a 9.4 [22]; alprenolol, pK_a 9.5 [22]; atenolol, pK_a 9.6 [22]; labetalol, pK_{a1} 7.4 [22], pK_{a2} 8.7 [22]; metoprolol, pK_a 9.7 [22]; nadolol, pK_a 9.39 [23]; pindolol, pK_a 9.7 [22]; propranolol, pK_a 9.5 [22].

3.1. Reduction of the original data set, visualization and selection of the BGEs to be tested

In Part I of this study, 222 possible background electrolytes (objects) were characterized by means of appropriate physico-chemical descriptors: pH, conductivity, ionic strength and relative viscosity [1]. The reduction of this original data set on the basis of pH values was necessary in order to consider only background electrolytes which make it possible the dissociation of the analytes [8–10,15] and thus to avoid unnecessary experiments. Hence, taking into consideration the basic nature of the analytes, the original data set was reduced to the BGEs with pH value less than or equal to 7, constituting a new subset of 117 objects. The new data matrix is reported in Table 1 and is constituted by 117 rows (objects) and 4 columns (descriptors).

After autoscaling the data, principal components were computed and on the basis of the K correlation index [24] the first two PCs (80.5% explained variance) were deemed able to describe the objects. PC3 retained 15.7% of the explained variance.

Loadings for PC1 were: pH, 0.15134; κ, 0.62394; μ, 0.64799; η, 0.40977. Loadings for PC2 were: pH, 0.84692; κ, −0.02321; μ, 0.14730; η, −0.51038. Examining the biplot of PC1 and PC2 (Fig. 1), the direction of conductivity and ionic strength was associated with PC1, pH was associated to PC2 and relative viscosity was associated to both components. Moreover, the dis-

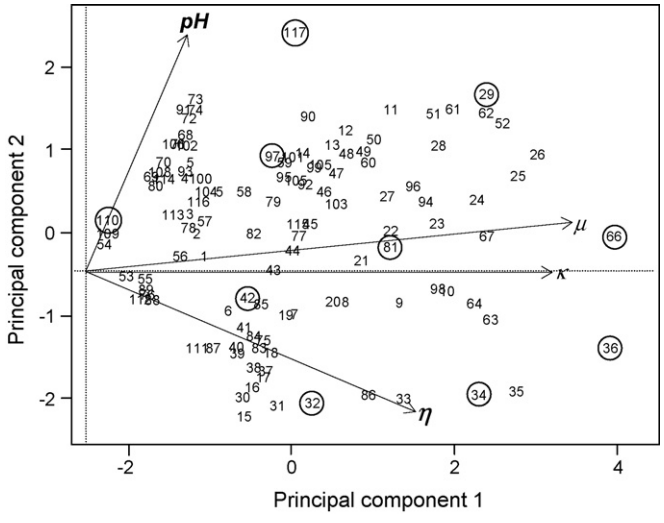

Fig. 1. Biplot on the first two principal components of 117 background electrolytes scores and loadings. Score axes are reported as abscissa and ordinate, loading axes are reported as dotted lines. Each index corresponds to a BGE (see Table 1). Variables are reported as: pH, κ (conductivity), μ (ionic strength), η (relative viscosity) and the arrows indicate the direction of the variables. The circles indicate the 10 BGEs to be tested in CZE, selected by means of Kennard–Stone algorithm.

Table 1
pH, conductivity (κ), ionic strength (μ) and relative viscosity (η) values of the selected background electrolytes with pH ≤ 7

	Background electrolyte, acronym when appropriate	pH	κ (mS cm^{-1})	μ (M)	η
1	W09A	3.93	1.80	0.020	1.019
2	W14A	4.12	1.75	0.020	1.015
3	W16A	4.29	1.69	0.020	1.012
4	W20A	4.88	1.53	0.020	1.009
5	W22A	5.25	1.430	0.020	1.011
6	W01B	3.65	1.475	0.020	1.032
7	W02B	3.97	2.67	0.040	1.039
8	W03B	4.21	3.90	0.060	1.040
9	W04B	4.39	5.02	0.080	1.044
10	W05B	4.55	6.11	0.100	1.044
11	CL06B	6.78	5.48	0.097	1.022
12	CL08B	6.35	4.71	0.075	1.020
13	CL09B	6.14	4.43	0.067	1.021
14	CL11B	5.74	4.05	0.057	1.017
15	BW01A	2.16	2.21	0.008	1.041
16	BW03A	2.69	1.64	0.019	1.041
17	BW04A	2.93	1.83	0.027	1.042
18	BW05A	3.13	2.09	0.034	1.038
19	BW07A	3.61	2.71	0.046	1.034
20	BW09A	4.04	3.35	0.066	1.038
21	BW11A	4.46	4.03	0.089	1.033
22	BW13A	4.81	4.60	0.108	1.031
23	BW15A	5.26	5.07	0.129	1.036
24	BW17A	5.70	5.50	0.155	1.037
25	BW19A	6.20	5.88	0.18	1.039
26	BW20A	6.66	6.04	0.189	1.040
27	BW01B	4.46	7.20	0.100	1.013
28	BW02B	5.90	7.48	0.114	1.020
29	BW05B	6.63	8.08	0.150	1.018
30	MI01A	2.22	2.11	0.018	1.037
31	MI02A	2.34	2.59	0.028	1.042
32	MI03A	2.49	3.13	0.045	1.044
33	MI05A	2.91	4.58	0.083	1.052
34	MI07A	3.27	5.86	0.114	1.058
35	MI08A	3.49	6.52	0.129	1.061
36	MI10A	4.16	7.75	0.190	1.062
37	BR01A	2.08	4.85	0.014	1.025
38	BR02A	2.13	4.16	0.016	1.026
39	BR05A	2.40	2.74	0.023	1.026
40	BR06A	2.57	2.47	0.025	1.027
41	BR08A	3.08	2.11	0.031	1.029
42	BR09A	3.59	2.10	0.034	1.028
43	BR11A	4.29	2.53	0.040	1.030
44	BR13A	4.72	2.85	0.046	1.031
45	BR15A	5.17	3.17	0.052	1.030
46	BR17A	5.83	3.53	0.057	1.030
47	BR18A	6.16	3.71	0.063	1.030
48	BR19A	6.47	3.89	0.069	1.029
49	BR20A	6.65	4.09	0.075	1.031
50	BR21A	6.85	4.24	0.080	1.031
51	K01A	6.05	7.55	0.124	1.013
52	K03A	6.53	7.92	0.151	1.025
53	Succinic acid A	2.84	0.601	0.002	1.008
54	Succinic acid B	3.06	0.265	0.000	1.001
55	K01B	3.11	0.489	0.004	1.013
56	K02B	3.73	0.968	0.014	1.016
57	K03B	4.39	1.62	0.026	1.016
58	K04B	4.97	2.36	0.042	1.017
59	K05B	5.59	3.01	0.061	1.017
60	S01B	5.93	5.28	0.080	1.024
61	S05B	6.81	6.42	0.133	1.024
62	S06B	6.98	6.71	0.147	1.029
63	S04C	1.92	11.49	0.130	1.014

Table 1 (*Continued*)

	Background electrolyte, acronym when appropriate	pH	κ (mS cm^{-1})	μ (M)	η
64	S05C	2.23	9.83	0.145	1.015
65	S06C	2.82	8.80	0.221	1.021
66	S07C	3.60	8.63	0.280	1.024
67	Na_2Hcitrate A	5.17	6.02	0.150	1.040
68	Na_2Hcitrate B	5.31	1.430	0.030	1.004
69	GV01A	4.73	0.763	0.010	1.006
70	GV02A	5.04	0.890	0.014	1.007
71	GV03A	5.41	1.153	0.020	1.007
72	GV04A	5.86	1.396	0.025	1.006
73	GV05A	6.22	1.440	0.028	1.006
74	P01A	6.92	0.371	0.006	1.019
75	H_3PO_4 A	1.93	5.93	0.016	1.014
76	H_3PO_4 B	2.16	1.99	0.006	1.004
77	KH_2PO_4 A	4.57	3.84	0.050	1.024
78	KH_2PO_4 B	4.60	0.852	0.010	1.020
79	$NH_4H_2PO_4$ A	4.59	3.76	0.050	1.014
80	$NH_4H_2PO_4$ B	4.64	0.861	0.010	1.008
81	KH_2PO_4/H_3PO_4 A	3.64	7.26	0.101	1.016
82	KH_2PO_4/H_3PO_4 B	3.60	3.91	0.051	1.009
83	H_3PO_4/KH_2PO_4 A	2.02	4.99	0.021	1.018
84	H_3PO_4/KH_2PO_4 B	2.25	4.08	0.03	1.019
85	H_3PO_4/KH_2PO_4 C	2.69	3.79	0.042	1.016
86	KH_2PO_4/H_3PO_4 C	2.40	5.7	0.056	1.041
87	KH_2PO_4/H_3PO_4 D	2.61	1.8	0.014	1.027
88	CH_3COOH A	3.02	0.321	0.000	1.018
89	CH_3COOH B	3.23	0.143	0.000	1.018
90	CH_3COONH_4 A	6.60	4.19	0.050	1.018
91	CH_3COONH_4 B	6.48	0.936	0.010	1.012
92	CH_3COONH_4/CH_3COOH A	5.49	3.91	0.050	1.022
93	CH_3COONH_4/CH_3COOH B	5.50	0.891	0.010	1.016
94	CH_3COONa/H_2SO_4 A	5.33	6.20	0.110	1.029
95	CH_3COONa/H_2SO_4 B	5.31	3.36	0.055	1.018
96	CH_3COONa/H_3PO_4 A	5.68	5.90	0.100	1.029
97	CH_3COONa/H_3PO_4 B	5.66	3.15	0.050	1.016
98	CH_3COONa/H_3PO_4 C	4.71	5.42	0.100	1.046
99	KCl A	5.40	5.42	0.050	1.015
100	KCl B	5.45	1.200	0.010	1.018
101	LiCl A	5.57	4.15	0.049	1.015
102	LiCl B	5.71	0.943	0.010	1.011
103	NaCl A	5.36	4.73	0.050	1.026
104	NaCl B	5.47	1.070	0.010	1.022
105	NH_4Cl A	5.29	5.65	0.050	1.013
106	NH_4Cl B	5.52	1.250	0.010	1.008
107	KNO_3 A	4.56	5.43	0.050	1.007
108	KNO_3 B	4.61	1.226	0.010	1.003
109	HCOOH A	3.17	0.245	0.003	1.000
110	HCOOH B	3.53	0.0664	0.001	1.001
111	Citric acid A	2.32	2.03	0.007	1.022
112	Citric acid B	2.58	0.883	0.003	1.011
113	H_3BO_3 A	4.94	0.004	0.000	1.021
114	H_3BO_3 B	5.43	0.004	0.000	1.017
115	TRIZMA HCl A	4.71	3.79	0.050	1.023
116	TRIZMA HCl B	5.18	0.868	0.010	1.021
117	TRIS CITRATE	6.68	0.624	0.155	1.001

tribution of electrolytes in the principal component space was not homogeneous. This irregularity was previously noticed, visualizing the objects in the corresponding biplot in the case of the application of the approach to the analysis of anti-inflammatory drugs, and was explained by the difficulty in having available electrolytes with all possible combinations of the variables [1].

Kennard–Stone algorithm [21] was then applied to the data matrix constituted by the 117 objects described by the scores on the first two principal components, that are significant according to *K* correlation index criterion [24], so it is better to use only the significant information (80.5% of the total variance) to select a set of BGEs excluding the noise and the useless information. The

10 selected BGEs, covering homogeneously the experimental space, are reported in Table 2 and their composition is described in the first part of this study [1]. Their position in the principal component space is shown in Fig. 1. The selected BGEs are made up by different buffering systems (citrate, phosphate, formate, acetate, glycine, borate) and are highly different in descriptors' values, covering all the range considered: pH, 2.49–6.68; κ, 0.066–8.63 mS cm^{-1}; μ, 0.001–0.280 M; η, 1.001–1.062.

3.2. Choice of a suitable BGE

In a screening phase, CZE analyses were carried out with the 10 selected BGEs, assuming that the others nearby in the plot would behave similarly [1,2]. The non-optimized experimental conditions 25 °C and 18 kV were applied, as these values were able to furnish a good compromise in keeping low both the generated current and the analysis time. The goal was to find the electrolyte which allowed the baseline resolution of the eight beta-blockers in a minimum analysis time.

Quite good results were obtained using phosphate buffer 81 (KH_2PO_4/H_3PO_4 A) and McIlvaine buffers 32 (MI03A) and 34 (MI07A), highlighted by squares in Fig. 2. However, with object 81 the resolution was poor for the peak pair alprenolol/propanolol, while for the other two electrolytes baseline disturbances were observed, especially with object 34. Thus, it was decided to go on with the investigation, reducing the data set around the three BGEs which gave better results. The area including these objects was enlarged, considering as borderline markers the scores of objects 84, 67 and 92 (Fig. 2). Selected scores for PC1 ranged from −0.54097 (BGE 84) to 2.3468 (BGE 67), while for PC2 the scores were limited to those lower than 0.60583 (BGE 92). Forty objects were included in this area and were maintained to carry on the study.

Subsequently, Kennard–Stone algorithm was applied to the new data set of 40 electrolytes described by the values of the original variables in order to identify another set of 10 electrolytes to be tested. The decision to use the original variables was made considering that in this step a visualization of the objects was not important, while a reduction of data handling could be attractive. The selected objects are reported in Table 3 and are highlighted by circles in Fig. 2.

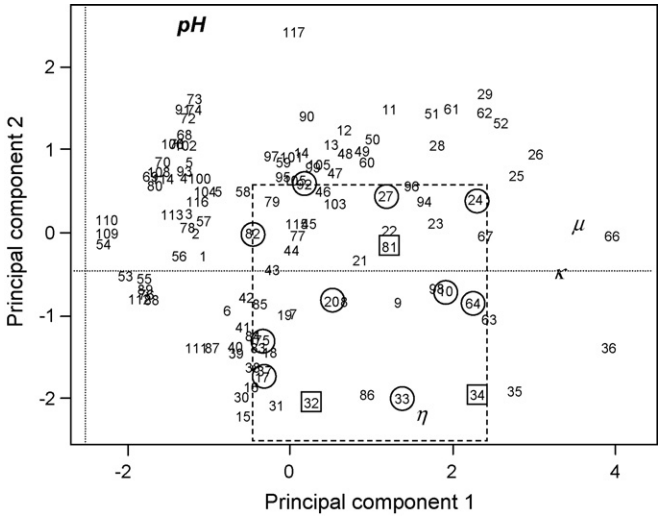

Fig. 2. Biplot on the first two principal components of 117 background electrolytes scores and loadings. Score and loading axes, variables and indexes as in Fig. 1. The dotted rectangle include the new experimental domain defined by the scores of objects no. 67, 84, 92. Objects no. 32, 34 and 81 (highlighted by squares) are the BGEs which in a first screening gave better results for the analysis of beta-blockers. The 10 objects highlighted with circles were selected by Kennard–Stone algorithm to continue the study.

Good results were obtained with several of these 10 BGEs. In particular, using object 20, a pH 4.04 Britton and Welford buffer (citric acid/NaOH), an electropherogram with baseline resolution among the eight beta-blockers was obtained in 10 min (Fig. 3a), thus this buffer was chosen as BGE. This object was very near object 8, consisting of a pH 4.21 Walpole buffer (acetic acid/sodium acetate), thus also this latter electrolyte was tested, but the results in terms of selectivity were poorer than using object 20. In particular, a lower resolution between alprenolol and propanolol was observed.

Buffer 20 was also compared with a BGE commonly used for the analysis of basic compounds, namely 50 mM pH 2.5 phosphate buffer. This latter showed almost the same analysis time (about 7% lower) but only six peaks were observed due to the complete overlap of two peak pairs, thus confirming the suitability of object 20 for analysing the test mixture.

Table 2
Ten BGEs selected by means of Kennard–Stone algorithm from the original data set of 117 electrolytes

	Background electrolyte	pH	κ (mS cm^{-1})	μ (M)	η
36	MI10A	4.16	7.75	0.190	1.062
110	HCOOHB	3.53	0.066	0.001	1.001
29	BW05B	6.63	8.08	0.150	1.018
32	MI03A	2.49	3.13	0.045	1.044
81	KH_2PO_4/H_3PO_4 A	3.64	7.26	0.101	1.016
117	TRIS CITRATE	6.68	0.624	0.155	1.001
97	CH_3COONa/H_3PO_4 B	3.53	0.066	0.001	1.001
66	S07C	3.60	8.63	0.280	1.024
42	BR09A	3.59	2.10	0.034	1.028
34	MI07A	3.27	5.86	0.114	1.058

Electrolytes are coded with the acronym reported in Part I of the study [1] and the index reported in Table 1.

Table 3
Ten BGEs selected by means of Kennard–Stone algorithm from the reduced data set of 40 electrolytes

	Background electrolyte	pH	κ (mS cm^{-1})	μ (M)	η
24	BW17A	5.70	5.50	0.155	1.037
75	H_3PO_4 A	1.93	5.93	0.016	1.014
64	S05C	2.23	9.83	0.145	1.015
33	MI05A	2.91	4.58	0.083	1.052
92	CH_3COONH_4/CH_3COOH A	5.49	3.91	0.050	1.022
27	BW01B	4.46	7.20	0.100	1.013
17	BW04A	2.93	1.83	0.027	1.042
82	KH_2PO_4/H_3PO_4 B	3.60	3.91	0.051	1.009
10	W05B	4.55	6.11	0.100	1.044
20	BW09A	4.04	3.35	0.066	1.038

Electrolytes are coded with the acronym reported in Part I of the study [1] and the index reported in Table 1.

3.3. Response surface methodology

After selecting citrate buffer pH 4.04 as background electrolyte, with the aim of further reducing analysis time while maintaining a baseline resolution among the peaks, a response surface study was carried out in the experimental domain defined by the instrumental parameters temperature and voltage. The considered factors were studied in the range 20–30 °C and 13–23 kV, respectively. This experimental domain was chosen in order to obtain a good description of the phenomenon in a wide zone of the factors' space, maintaining a good compromise between generated current and analysis time. It was deemed sufficient to take into consideration only the instrumental parameters, in fact, having already obtained the baseline resolution of the analytes, the main purpose of the response surface study was an improvement of migration time.

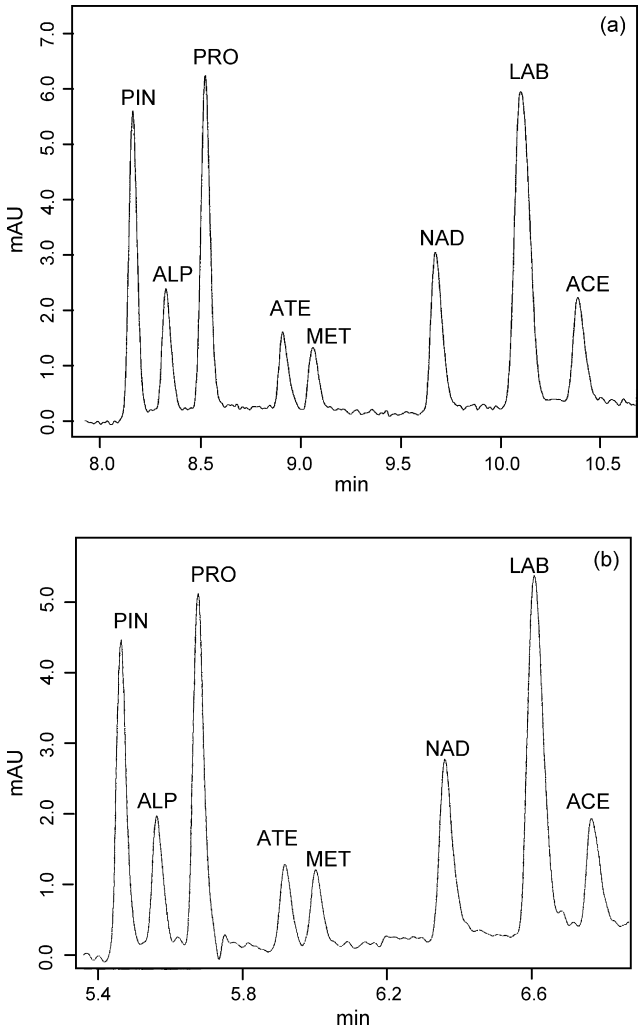

Fig. 3. Electropherogram of the beta-blocker drugs. Background electrolyte: object 20-BW09A, pH 4.04, $\kappa = 3.35$ mS cm^{-1}, $\mu = 0.066$ M, $\eta = 1.038$—(a) non-optimized conditions: voltage 18 kV, temperature 25 °C; (b) optimized conditions: voltage 23 kV, temperature 26 °C.

The considered responses were the critical resolution R_4 (ATE/MET) and analysis time (t), calculated as migration time of the last peak.

The response surfaces were estimated by running a Doehlert design [5] with nine experiments. R^2 and Q^2 for the different calculated models were: R_4, $R^2 = 0.924$, $Q^2 = 0.624$; t, $R^2 = 0.985$, $Q^2 = 0.481$.

The response surfaces obtained indicated that for maximizing R_4 voltage should be set at high level and temperature at low level, evidencing a negative interaction between these two factors. Analysis time was minimised by low levels of both factors.

Desirability function [5] provided convenient means to select the optimum with the most desirable properties. In order to achieve a baseline resolution for all the peaks, the target value for the critical resolution R_4 was 1.5. The fully desired analysis time was defined below 7 min, with partially accepted values between 9 and 7 min. The optimized conditions corresponded to voltage, 23 kV, and temperature, 26 °C. Applying these conditions, the separation of the eight beta-blockers was obtained in about 7 min (Fig. 3b), which is about 40% lower than that previously reported for a similar application [25].

4. Conclusions

Successful results were obtained with both the applications presented in the two parts of the study, showing the reliability and versatility of the presented chemometric approach for the selection of a suitable background electrolyte. Each analytical problem presents a different electrophoretic behaviour that has to be evaluated, but this rational approach has general utilization

and makes it possible to save time and costs associated with the background electrolyte selection phase of method development. The only precaution is the reduction of the initial data set to an appropriate pH range for the specific application, which is important in order to avoid useless experiments. Furthermore, this reduction could also be made on the basis of conductivity, in addition to pH. This can be especially useful for MEKC and MEEKC analysis, where high values of conductivity can give rise to problems related to high-generated currents.

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