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ORIGINAL

Multivariate Optimization and Validation of a CZE Method for the Analysis of Pridinol Mesylate and Meloxicam in Tablets

Silvana E. Vignaduzzo · Luciana Vera-Candioti · Patricia M. Castellano · Héctor C. Goicoechea · Teodoro S. Kaufman

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Abstract A capillary zone electrophoresis method for the simultaneous determination of pridinol mesylate (PRI) and meloxicam (MEL) employing epinastine hydrochloride and piroxicam as internal standards, was developed and optimized employing experimental design and response surface methodologies. The separation was optimally achieved in less than 2 min at 30 kV in an uncoated fused-silica capillary (41.4 cm × 75 μm I.D.), employing an 18 mmol L^{-1} sodium phosphate buffer solution (pH 5.90) at 25 °C. Samples were injected in hydrodynamic mode (50 mbar, 5 s) and the analytes were spectrophotometrically detected at 200 nm. Method robustness was demonstrated by ANOVA of determinations performed under conditions slightly different from the optimum. The method was validated regarding separation selectivity (peak purity factors > 0.99), linearity and range (PRI = 17.6–31.4 mg L^{-1}; MEL = 66.5–122.5 mg L^{-1}), accuracy (PRI = 100.2–101.9%; MEL = 98.9–100.7%) and precision. The RSD values obtained were ≤1.3% for injection repeatability and ≤1.9% for intra-day precision. The limits of detection (1.0 and 0.9 mg L^{-1}) and quantification (3.3 and 16.5 mg L^{-1}) of PRI and MEL, respectively, were also determined. The method was successfully applied to the determination of both drugs in three brands of tablet formulations. No statistically significant differences were observed when these results were compared with those of a RP-HPLC method.

Keywords Capillary zone electrophoresis · Experimental designs · Meloxicam · Pridinol

S. E. Vignaduzzo · P. M. Castellano · T. S. Kaufman (✉)
Pharmaceutical Analysis, Department of Organic Chemistry, Faculty of Pharmaceutical and Biochemical Sciences, National University of Rosario and Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), Suipacha 531, S2002LRK Rosario, Argentina
e-mail: kaufman@iquir-conicet.gov.ar

L. Vera-Candioti · H. C. Goicoechea (✉)
Laboratory of Analytical Development and Chemometrics (LADAQ), Analytical Chemistry I, Faculty of Biochemistry and Biological Sciences, National University of the Litoral, University City, S3000ZAA Santa Fe, Argentina
e-mail: hgoico@fbcb.unl.edu.ar

Introduction

Pridinol mesylate (PRI, Fig. 1) is a central anticholinergic [1], employed as a myotonolytic and spasmolytic agent in antistress therapy [2] and for the treatment of Parkinson's disease [3–5]. Meloxicam (MEL) is a non-steroidal anti-inflammatory drug [6, 7]. The combination therapy of pridinol and meloxicam is prescribed as anti-inflammatory, analgesic and myorelaxant, being especially useful for treating muscular contractures and low back pains [8–10]. We have recently reported chromatographic determinations of PRI in the presence of its impurities [11, 12] and other drugs [13], and a RP-HPLC method for the simultaneous determination of PRI and MEL in tablets [14].

In recent years, capillary electrophoresis (CE) has notably expanded its scope and range in aspects of instrumentation and applications, still being a rapidly growing technique [15]. Although CE is official in the leading Pharmacopoeias, only a few applications are currently included [16, 17]. However, there are clear prospects of increasing impact of this methodology in modern pharmaceutical analysis [18–20].

Capillary electrophoretic methodologies have been reported for the determination of PRI [21] and MEL [22] alone and for MEL in mixtures with other analytes [23];

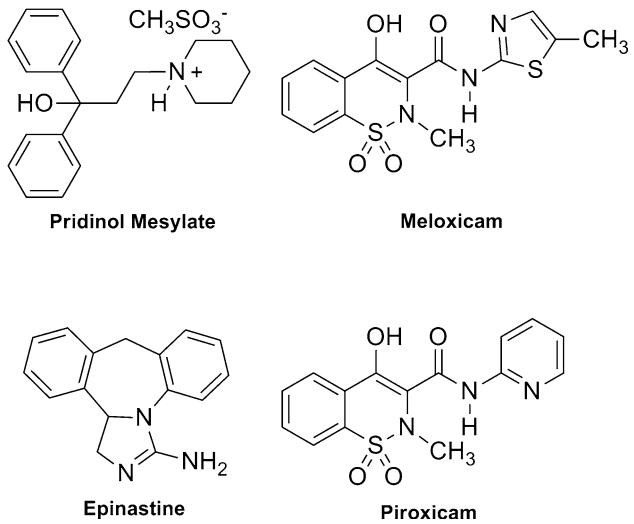

Fig. 1 Chemical structures of meloxicam, pridinol mesylate and their corresponding internal standards Epinastine and Piroxicam

however, none of the reported CE methods considered their simultaneous quantification. Therefore, herein we report the development of a simple and rapid CZE method for the simultaneous determination of the PRI and MEL, using experimental design strategies, and its validation with regard to the parameters included in the ICH validation guideline [24]. The successful application of the validated method to the determination of both drugs in their combined tablet formulations and comparison of its performance with the published HPLC [14] method are also reported.

Experimental

Instrumentation

All the CE experiments were carried out on an Agilent ^{3D}CE capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), equipped with an on-column diode array detector and an automatic injector. The separations were carried out in normal mode, employing a 41.5 cm (effective length = 33 cm) × 75 µm I.D. uncoated fused-silica capillary (MicroSolv Technology Corporation, Eatontown, USA), applying a potential of 30 kV, with a typical current of 80.0 (±0.2) µA. The capillary was thermostated at 25 °C. Samples were introduced hydrodynamically, applying 50 mbar of pressure for 5 s and kept at room temperature in the autosampler. For improved performance, detection of the analytes was performed at 200 nm and spectra were collected every 2 nm between 200 and 280 nm for peak purity determinations.

The RP-HPLC separations were performed with a Varian Prostar 210 liquid chromatograph (Varian Inc., Walnut Creek, USA) consisting of two pumps, a manual injector fitted with a 20 µL loop and a Varian Prostar 325 variable-wavelength UV–visible detector set at 225 nm. Compounds were separated on a 250 × 4.6 mm C_{18} column (Luna, Phenomenex, 5 µm particle size), employing a 51:9:40 (*v/v/v*) mixture of methanol, 2-propanol and 50 mmol L^{-1} potassium phosphate buffer (pH 5.9) as mobile phase, pumped at a flow rate of 1.0 mL min^{-1} [14].

The pH of the solutions was determined employing a Hanna pH-meter (Hanna Instruments, Inc., Woonsocket, USA). All solutions were degassed in a Cole Palmer 8891 ultrasonic bath (Cole Palmer, IL, USA) and filtered through 0.45-µm Nylon membranes (Sartorius AG, Goettingen, Germany) before use. It should be noted that at pH = 5.9, the background electrolyte almost has no buffer capacity. On the other hand, ten analyses were performed with a buffer vial before exchanging the electrolyte solution.

Software

The CE ChemStation software (Agilent Technologies) was employed for the CE instrument control and data acquisition. The chromatograms were recorded and analyzed employing Varian Star v. 6.4 software. Experimental design, data analysis, and desirability function calculations were performed with the aid of Design-Expert 7.0.3 (Stat-Ease, Inc., Minneapolis, USA). Statistical analyses were carried out with Statgraphics Plus 5.1 (Statpoint Technologies, Inc., Warrenton, USA).

Chemicals and Reagents

Sodium dihydrogen phosphate, sodium hydroxide and acetonitrile were obtained from Merck (Darmstadt, Germany). Pharmaceutical grade PRI and MEL were provided by Droguería Saporiti (Buenos Aires, Argentina). The standards of epinastine hydrochloride (EPI) and piroxicam (PIR) were obtained from Boehringer Ingelheim and Sigma-Aldrich, respectively. HPLC-grade methanol and 2-propanol were acquired from Mallinckrodt-Baker (Phillipsburg, USA). Milli-Q quality ultra-pure water was used in all the experiments. All other reagents were of analytical grade. Tablets containing MEL and PRI, labeled to contain 15 mg MEL and 4 mg PRI, were acquired from local pharmacies.

Standard and Sample Solutions

Background Electrolyte Solutions

Several background electrolyte (BGE) buffers, prepared by appropriate dilution of a 0.1 mol L^{-1} stock solution of sodium dihydrogen phosphate, adjusting the pH with

0.1 mol L^{-1} NaOH and filling up to 100 mL with water, were examined during the optimization stage. In the optimized procedure, the BGE was an 18 mmol L^{-1} sodium phosphate solution of pH 5.90. The solutions were prepared daily; they were filtered through 0.45-µm Nylon membranes (Sartorius AG, Germany) and degassed before use.

Standard Solutions for CZE Analysis

Stock standard solutions of MEL (700 mg L^{-1}), PRI (1,960 mg L^{-1}), EPI (2,000 mg L^{-1}) and PIR (1,960 mg L^{-1}) were individually prepared in 100 mL volumetric flasks by dissolving with a 1:1 (*v/v*) acetonitrile–water mixture, accurately weighed amounts of the corresponding drugs and diluting to the mark with the solvent. These solutions were stored at 4 °C in the dark.

For screening and optimization purposes, a mixed standard solution containing 24.5 mg L^{-1} PRI, 94.5 mg L^{-1} MEL, 20.0 mg L^{-1} EPI and 97.0 mg L^{-1} PIR, prepared by mixing appropriate volumes of the corresponding working standard solutions and diluting with a 1:1 (*v/v*) mixture of acetonitrile–water, was employed. Mixed standard solutions for validation purposes were prepared analogously. All the solutions were prepared daily, in volumetric flasks, and the experiments were performed in random order.

Tablet Samples

For the analysis of the dosage form, 20 tablets were weighed and finely powdered in a mortar. An accurately weighed portion of the powder was transferred into a 10 mL volumetric flask using 5 mL of a 4:1 (*v/v*) mixture of methanol and 0.1 mol L^{-1} NaOH. The flask was submitted to ultrasonic treatment for 10 min, when methanol was added to the mark.

After centrifugation (10 min at 1,900×*g*) to remove minor amounts of insoluble matter, 0.30 mL of the supernatant was transferred to a 2.0 mL volumetric flask, appropriate volumes of the stock solutions of the internal standards were added and the mixture was diluted to the mark with acetonitrile–water (1:1, *v/v*), to yield approximate final concentrations of 24.5 mg L^{-1} for PRI, 20.0 mg L^{-1} for EPI, 94.5 mg L^{-1} for MEL and 97.0 mg L^{-1} for PIR. Before being used, the solutions were filtered through 0.45-µm Nylon membrane filters (Sartorius, Germany). Three aliquots of tablet powder were similarly processed for each commercial brand.

Electrophoretic Technique

To obtain reproducible results, careful activation and conditioning of the capillary was found to be relevant. At the beginning of every working day, it was successively rinsed (10 min each) with 0.1 mol L^{-1} NaOH, water and BGE. Between runs, the capillary was successively flushed (3 min each) with 0.1 mol L^{-1} NaOH, water and BGE. At the end of the day, the capillary was washed with 0.1 mol L^{-1} NaOH (5 min) and water (5 min), and then air-dried for 3 min.

Results and Discussion

Method Development

Internal Standards

One of the most common shortcomings of electrophoretic analysis is the poor reproducibility of the migration times of the analytes and their peak areas. The main reason for this drawback is the variation in electroosmotic flow, mainly due to a non-reproducible inner capillary wall. This can be significantly improved by consistent capillary washing and resorting to the use of internal standards.

Preliminary experiments confirmed the benefits of an internal standard. However, due to the marked differences in the properties of MEL and PRI, it was also observed that using a single internal standard did not afford accurate and precise determinations of both analytes. Therefore, two internal standards were included for method development; this strategy has been employed in different separation settings including HPLC [25, 26], GC [27] and CE [28], for improving method performance.

Epinastin and piroxicam (Fig. 1) were selected as the internal standards for PRI and MEL, respectively. These drugs have similar physicochemical properties (structure, pKa and solubility) and detector responses to those of the corresponding analytes of interest. In addition, they are stable and chemically inert toward the other components of the samples.

Screening of Relevant Factors for Method Optimization

Concentration and pH of the background electrolyte and instrumental settings such as capillary temperature and separation voltage can all significantly influence CZE analyses. Moreover, these parameters are often interactive in nature and, therefore, should be incorporated in the method development strategy.

Therefore, an initial screening was conducted with a mixed standard solution, to determine the most significant factors affecting five responses [the resolutions between the peaks of EPI and PRI ($R_{s\text{-EPI/PRI}}$) and between the peaks of MEL and PIR ($R_{s\text{-PIR/MEL}}$), the analysis time (t), peak width of MEL (w_{MEL}) and current intensity (I) within the

capillary]. The experimental domain (temperature 25–30 °C; separation voltage 25–30 kV; BGE pH 5.0–6.0; BGE concentration 15–25 mmol L^{-1}) was selected upon the knowledge of the system acquired from trial and error runs, with experiments arranged according to a 2^4 full factorial experimental design strategy, with six replicates of the central point [29].

The subsequent ANOVA of the responses revealed that: capillary temperature and separation voltage did not affect significantly the resolution between the analytes and their corresponding internal standards; capillary temperature had no influence on the separation time; buffer composition significantly affected all of the studied responses ($p < 0.05$). Therefore, for the optimization stage the capillary temperature was set at 25 °C and the separation voltage was established at 30 kV, aiming to minimize the analysis time.

Method Optimization

Experiments were carried out under different conditions according to a central composite design, which included combinations of BGE concentration (13–27 mmol L^{-1}) and BGE pH (4.80–6.20), and four replicates of the central point (Table 1). These ranges were selected based on prior knowledge about the system under study.

The experimental data of the five responses were fitted by polynomial models in which coefficients were

Table 1 Full-Factorial 2^4 design built for the factor selection and results of the ANOVA test on the responses

Run No.	Factors				Responses				
	Buffer (mmol L^{-1})	pH	V (kV)	Temp. (°C)	$R^{a}_{s\text{-PRI/EPI}}$	$R^{a}_{s\text{-MEL/PIR}}$	t (min)	Current (μA)	w_{MEL} (min)
1	20.00	5.5	27.5	27.5	1.66	3.20	2.013	76.0	0.170
2	15.00	6.0	30.0	30.0	1.52	1.88	1.484	69.0	0.097
3	15.00	6.0	25.0	25.0	1.76	2.10	2.074	48.5	0.142
4	20.00	5.5	27.5	27.5	1.76	2.80	2.000	76.0	0.174
5	15.00	5.0	25.0	25.0	1.80	3.94	2.400	48.0	0.220
6	20.00	5.5	27.5	27.5	1.84	3.76	1.987	80.3	0.175
7	25.00	6.0	25.0	25.0	2.04	2.06	2.549	90.6	0.220
8	20.00	5.5	27.5	27.5	1.82	3.78	1.956	80.4	0.158
9	25.00	5.0	25.0	25.0	2.10	3.98	5.991	85.0	2.400
10	25.00	6.0	30.0	25.0	2.00	1.84	2.373	130.0	0.310
11	15.00	6.0	25.0	30.0	1.52	1.86	1.775	52.5	0.108
12	25.00	5.0	30.0	25.0	2.34	5.44	2.929	120.0	0.460
13	25.00	5.0	30.0	30.0	2.16	5.06	1.773	130.0	0.152
14	25.00	5.0	25.0	30.0	2.40	5.82	2.573	92.0	0.255
15	20.00	5.5	27.5	27.5	2.00	4.08	2.045	80.0	0.166
16	15.00	5.0	25.0	30.0	1.64	4.56	1.963	51.0	0.165
17	15.00	5.0	30.0	30.0	1.72	4.36	1.691	66.8	0.167
18	20.00	5.5	27.5	27.5	1.94	3.80	2.124	80.0	0.195
19	15.00	6.0	30.0	25.0	1.38	1.92	1.560	64.0	0.100
20	25.00	6.0	25.0	30.0	2.32	1.98	2.383	98.0	0.212
21	15.00	5.0	30.0	25.0	1.76	4.58	1.849	62.7	0.172
22	25.00	6.0	30.0	30.0	1.62	1.64	1.598	144.0	0.125
			Probability value[b]						
Model[c]			<0.0001	<0.0001	0.0033	<0.0001	<0.0001		
Buffer concentration (mmol L^{-1})			0.0001 (+)	>0.05	0.0119 (+)	0.0001 (+)	0.0001 (+)		
pH			0.0318 (−)	0.0001 (−)	>0.05	0.0002 (+)	0.0001 (−)		
Separation voltage (kV)			>0.05	>0.05	0.0246 (−)	0.0001 (+)	0.0154 (−)		
Temperature (°C)			>0.05	>0.05	0.0033 (+)	0.0001 (+)	0.0010 (−)		

[a] Resolution between analytes

[b] Considered significant when $p < 0.05$. Signs between parentheses correspond to the effects on the variables

[c] Statistical significance of the linear model

computed by stepwise backward multiple regression and validated by ANOVA tests. The analysis time, $R_{s\text{-EPI/PRI}}$ and the capillary current were fitted by linear models, while $R_{s\text{-PIR/MEL}}$ and w_{MEL} required quadratic models. In order to reach a compromise among the responses which could better satisfy the objectives, the fitted responses were simultaneously optimized employing Derringer's desirability function. This is a response surface methodology which allows mapping the degree of compliance of a system's response with user-defined conditions, and to predict its responses within the experimental domain [30].

This approach to the simultaneous optimization of multiple responses involves creating a partial desirability function (d_i) for each of the n individual responses, in which values range between 0 for a fully undesirable response and 1 for a completely desirable response. The partial desirabilities are then combined into a global desirability function D, computed as the geometric mean of the partial desirabilities ($D = n^{-1}\ [\Pi\ (d_{i\ =\ 1,\ldots,n})]^{1/n}$, the maximum of which should yield the conditions of the designed variables for an optimum response.

The goals set were to minimize the analysis time and w_{MEL} while maximizing $R_{s\text{-PRI/EPI}}$, keeping $R_{s\text{-MEL/PIR}}$ >1.50 and maintaining the capillary current below 100 μA. Under these optimization criteria, a maximum of the desirability function ($D = 0.53$) was found at BGE concentration = 18.0 mmol L^{-1} and BGE pH = 5.90 (Fig. 2). Under these conditions, it was predicted baseline resolutions between the MEL, PRI and their corresponding internal standards, in short analysis time ($t = 1.67$ min), at a capillary current of 80 (± 0.2) μA, where $w_{\text{MEL}} = 0.11$ min. The predicted optimum values were experimentally corroborated, yielding the typical electropherogram shown in Fig. 3. The pK_a values of the analytes are as follows: PRI (9.7) [31], EPI (11.2) [32], MEL (1.09 and 4.18) [31] and PIR (1.86 and 5.46) [33]; they explain the observed separation order. Under the separation conditions (pH = 5.90), PRI and EPI are positively charged; therefore, they are the first pair of analytes appearing in the electropherogram. Then, the signal of the uncharged electro-osmotic flow (EOF) is observed, followed by the peaks of the negatively charged MEL and PIR. On the other hand, the small peak at ~0.6 min can be attributed to an impurity.

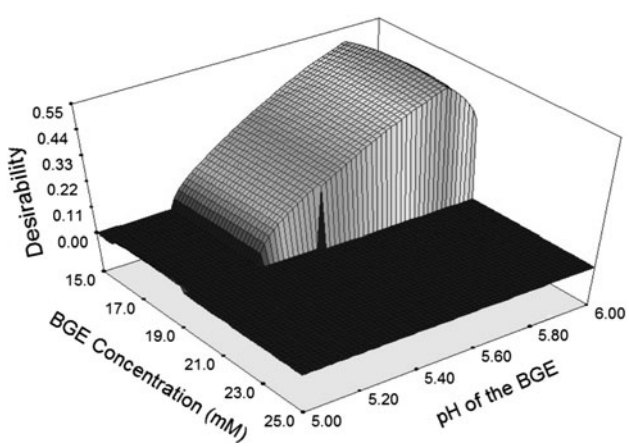

Fig. 2 Response surface plot corresponding to the desirability function of the separation, when optimizing concentration and pH of the background electrolyte. Separation voltage = 30 kV; capillary temperature = 25 °C

Method Robustness

Method parameters such as the capillary temperature and the applied voltage are very well controlled by the instrument software and were shown not to affect resolution between adjacent peaks. However, concentration and pH of the BGE are the most important parameters that are likely suffer variations, with potential impact on the analytical performance of the method.

The influence of these variables on the method was studied with a tablet sample, through the evaluation of the effect in small variations of BGE concentration (2.0 mmol L^{-1}) and pH (0.2 pH units), following a 2^2 factorial design. Under each condition, samples were analyzed in triplicate, resolutions and recoveries of the analytes were recorded and the effects of the modifications were statistically evaluated by means of an ANOVA test.

Overall analytes' recoveries were 101.1 ± 1.6 and 99.0 ± 1.4% for PRI and MEL, respectively, and the

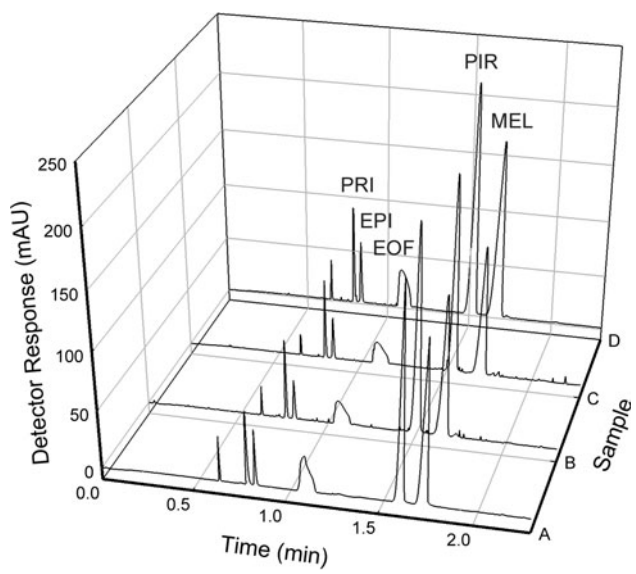

Fig. 3 Typical electropherograms corresponding to different samples under the optimized experimental conditions. **a** Standard solution containing 24.5 mg L^{-1} PRI, 20 mg L^{-1} EPI, 94.5 mg L^{-1} MEL and 97 mg L^{-1} PIR in a 1:1 (*v/v*) mixture of acetonitrile–water. **b–d** Commercial tablet samples of brands 1–3, respectively

ANOVA revealed that the above changes performed in concentration ($F_{PRI} = 0.30$; $F_{MEL} = 0.18$) and pH ($F_{PRI} = 0.07$; $F_{MEL} = 2.86$) of the background electrolyte did not have a statistically significant effect ($F_{0.95,\ 1,\ 9} = 5.12$) on the recoveries of the analytes, confirming method robustness. On the other hand, the critical resolution between PRI and EPI ($R_{s\text{-PRI/EPI}}$) remained satisfactory, above 1.6.

Method Validation

The developed method was validated employing mixed standard solutions and tablet samples, with respect to the following parameters, described in the ICH guidelines: specificity, range and linearity, inter- and intra- assay precision and accuracy. Limits of detection and quantification were also determined for both analytes.

Separation Selectivity

According to the ICH Q2(R1) guideline, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. However, the IUPAC discourages the use of this term [34], being usually replaced by separation selectivity in electrophoretic techniques. Aiming to verify that the peaks of PRI and MEL correspond to pure compounds, purity tests based on the correlation between the spectra of the components recorded within the peaks were carried out. The analyzed peaks yielded purity factors greater than the established threshold limit (0.99) in mixed standard solutions and in tablet samples, confirming that the excipients do not interfere with the determination.

Range and Linearity

Method linearity was verified by analysis of triplicate injections of mixed standard solutions of PRI (17.6–31.4 mg L^{-1}) and MEL (66.5–122.5 mg L^{-1}) at five concentration levels, containing EPI (20.0 mg L^{-1}) and PIR (97.0 mg L^{-1}) as internal standards, which were subjected to the optimized analytical procedure. The studied concentrations covered the range of 70–130% with regard to the nominal test concentrations of the analytes in the tablet preparations.

Excellent correlations ($r > 0.99$) were obtained when the peak-area ratios for each analyte and its corresponding internal standard were plotted against the corresponding nominal concentrations of the analyte and the data were linearly fitted by the least-squares method (Table 2). The homoscedasticity of the data was evaluated trough an F test of the variances at the lower and upper limit of range. The difference between the observed and the critical value of F was not significant ($\alpha = 0.05$). The lack of fit to linear model was also evaluated by an ANOVA test as suggested by IUPAC [35] with satisfactory results.

This confirmed that the response linearity was suitable for the entire assay range.

Accuracy

Demonstration of method accuracy was carried out by recovery experiments, employing a solution (100 µL) of a pre-analyzed tablet sample, containing the analytes at a level equivalent to 30% of the label claim and the internal standards. This solution was spiked with known amounts of PRI and MEL in order to yield quality control solutions with analyte concentrations corresponding to 80, 100 and

Table 2 Central composite design used for the optimization of five responses

Run No.	Factors		Responses				
	Buffer conc. (mmol L^{-1})	pH	$R_{s\text{-PRI/EPI}}$	$R_{s\text{-MEL/PIR}}$	t (min)	Current (µA)	w_{MEL} (min)
1	20.0	4.79	2.30	5.62	2.443	103.0	0.280
2	20.0	5.50	1.75	5.42	1.754	88.0	0.140
3	20.0	5.50	1.88	5.34	1.756	88.0	0.145
4	20.0	5.50	2.06	5.30	1.841	88.0	0.155
5	15.0	6.00	1.52	1.77	1.429	68.5	0.090
6	15.0	5.00	2.03	6.05	2.116	62.5	0.285
7	20.0	5.50	1.77	4.96	1.684	92.5	0.143
8	20.0	6.21	1.92	2.10	1.857	94.0	0.170
9	25.0	6.00	1.98	2.14	1.639	120.0	0.125
10	20.0	5.50	1.71	5.65	1.625	92.5	0.118
11	25.0	5.00	2.02	5.84	1.741	120.0	0.125
12	12.9	5.50	1.79	5.89	1.694	53.0	0.114
13	27.1	5.50	2.44	5.32	2.244	136.0	0.255

120% of the label claim, which were analyzed by performing six replicates.

The results, obtained by means of the regression line equation developed in the linearity experiment, showed almost quantitative average recovery values (Table 2). Considering the requirements for bioanalytical assays, these results were indicative of the high method accuracy obtained at the three concentration levels studied.

Precision

The intra- and inter-day precisions were assessed. The intra-day precision was determined by analysis of six independent samples of tablets of Brand No. 3 at the expected concentration level (100%) of the analytes. Observed RSD values were 1.3% for PRI and 1.0% for MEL, indicating that method precision is satisfactory.

On the other hand, the inter-day precision of the method was determined by triplicate analysis of independent samples of the same brand of tablets, run in groups of six under the optimum conditions by the same analyst, during three different days. No statistically significant differences in method performance during the different days ($p < 0.05$) were observed when the results were submitted to an ANOVA test. Overall mean drug recoveries were 101.7 ± 1.6 and 98.8 ± 1.2% for PRI and MEL, respectively (Table 2). These results confirmed that the method is precise.

Limits of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were computed for both analytes based on the signal-to-noise ratios (S/N) [36]. The LOD values were 1.0 and 0.9 mg L^{-1} for PRI and MEL, respectively, being 3.3 and 16.5 mg L^{-1} the corresponding LOQ results (Table 3).

System Suitability

Based on multiple experiments, the critical resolution between PRI and EPI ($R_{s\text{-PRI/EPI}} > 1.5$) and the RSD of replicate ($n \geq 6$) injections (PRI = 1.7%; MEL = 1.3%) of the mixed standard solution with the analytes at the 100% level were selected as performance parameters to be met by the system prior to analysis.

Stability of the Solutions

The results of peak area determination of working samples stored for 24 h at room temperature showed that they are stable for at least this time. Similarly, the stock standard solutions proved to be stable for at least 3 months at 4 °C.

Table 3 Main validation results for the proposed method

Validation parameter	Analyte	
	PRI	MEL
Separation selectivity (peak purity factor)	>0.99 (complies)	>0.99 (complies)
Range (mg L^{-1})	17.6–31.4	66.5–122.5
Linearity [PAR = $a + bx$ (mg L^{-1})]		
Calibration solutions (mg L^{-1})	17.6; 21.0; 24.5; 28.0; 31.4	66.5; 80.5; 94.5; 108.5; 122.5
r ($n = 15$)	0.9947	0.9911
Intercept (a) ±SD	0.04 ± 0.01	−0.002 ± 0.010
Slope (b) ±SD	0.0272 ± 0.0004	0.0103 ± 0.0002
Accuracy. Added and recovered [% (mg L^{-1}), $n = 6$]		
80% level (PRI = 10.78, MEL = 42.35)	101.3 ± 1.9	100.7 ± 0.5
100% level (PRI = 15.68, MEL = 61.25)	101.9 ± 1.7	98.9 ± 1.0
120% level (PRI = 20.58, MEL = 80.15)	100.2 ± 1.2	99.8 ± 1.3
Precision		
Repeatability (CV,%); $n = 6$	1.3	1.0
Intermediate precision [mean ± RSD (%)]		
Day 1; $n = 18$	101.7 ± 1.5	99.0 ± 1.3
Day 2; $n = 18$	101.7 ± 1.9	98.9 ± 0.9
Day 3; $n = 18$	101.3 ± 1.0	98.7 ± 1.3
Overall recovery; $n = 54$	101.7 ± 1.6	98.8 ± 1.2
F-days ($F_{0.95,\ 2,\ 46} = 3.20$)	0.01	0.89
F-samples ($F_{0.95,\ 5,\ 46} = 2.42$)	1.81	2.12
LOD (mg L^{-1})	1.0	3.3
LOQ (mg L^{-1})	0.9	16.5

Table 4 Application and method comparison

Method	Replicate	Brand 1		Brand 2		Brand 3	
		PRI	MEL	PRI	MEL	PRI	MEL
CZE	1	102.9	99.1	99.5	98.0	100.4	98.8
	2	102.4	101.6	99.4	97.1	100.5	99.6
	3	101.2	101.1	99.2	97.3	101.6	97.9
	Average	102.2	100.6	99.4	97.5	100.8	98.8
	RSD (%)	0.9	1.3	0.2	0.5	0.7	0.9
HPLC	1	101.9	98.9	100.1	98.1	101.3	99.4
	2	102.7	101.8	98.8	97.9	98.7	98.9
	3	103.1	100.1	99.8	98.6	100.9	100.1
	Average	102.6	100.3	99.6	98.2	100.4	99.5
	RSD (%)	0.6	1.5	0.7	0.4	1.4	0.6
t_{calc} ($t_{crit} = 2.77$)		0.66	0.28	0.48	1.91	0.45	1.15

Results obtained from three different commercial tablet samples

Application. Assay of PRI-MEL Pharmaceutical Formulations

Three different commercial tablet brands of the PRI-MEL pharmaceutical association (Table 3) were analyzed. All the samples complied with the typical official specification for tablets requiring them to contain not less than 90% and not more than 110% of the labelled amount of the active principles [15]. Figure 3 exhibits typical electropherograms recorded for samples of tablets of the different brands.

Comparison with HPLC

No statistically significant differences were found (Table 4) when the contents of both drugs in the tablet samples were determined by a validated HPLC method [14].

Conclusions

A simple, robust, rapid and convenient CZE method for the simultaneous determination of PRI and MEL was developed and optimized employing experimental design and surface response methodologies.

Method reliability was assessed against requirements of international regulations for the quality control of pharmaceuticals. Satisfactory calibration linearity in the working ranges, repeatability of peak-area ratios and inter-day precision results were obtained. The proposed method also demonstrated to fulfill pre-established requirements for separation selectivity, accuracy and LOQ, being confirmed as suitable for its intended purpose.

The CZE method was successfully applied to the determination of PRI and MEL in their combined tablet formulations, yielding results not statistically different from those provided by the published HPLC procedure. However, low-cost, non-polluting conditions, high sample throughput and minimum requirements for sample pretreatment, are interesting advantages of the CZE determination, making it amenable for routine use.

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