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Method development and validation for the simultaneous determination of cinnarizine and co-formulated drugs in pharmaceutical preparations by capillary electrophoresis

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Abstract

Rapid and simple capillary electrophoresis (CE) methods were developed for the simultaneous determinations of cinnarizine and domperidone (CN/DOM) and cinnarizine and nicergoline (CN/NIC) in their co-formulated tablets. The optimized CE conditions were as follows: running buffer, methanol–acetate buffer (pH 3.0, 10 mM) (80:20 and 85:15 (v/v) for CN/DOM and CN/NIC, respectively); applied voltage, 20 kV; UV detection wavelengths, 215 and 227 nm for CN/DOM and CN/NIC, respectively; hydrodynamic injection was performed at a height of 25 mm for 30 s. Quinine hydrochloride and nicardipine hydrochloride were used as internal standards for the determination of CN/DOM and CN/NIC, respectively. Calibration curves were linear over the ranges 0.25–20/0.375–15 μg/ml (CN/DOM) and 0.25–25/0.4–10 μg/ml (CN/NIC) in each optimized condition. Detection limits were 0.074/0.119 μg/ml and 0.072/0.116 μg/ml for CN/DOM and CN/NIC, respectively. The proposed methods were successfully applied for the simultaneous determination of both CN/DOM and CN/NIC in their co-formulated tablets without interfering peaks due to the excipients present in the pharmaceutical tablets. The estimated amounts of CN/DOM and CN/NIC were almost identical with the certified values, and their percentage relative standard deviation values (%R.S.D.) were found to be ≤2.34% ($n = 3$).

Keywords: Capillary electrophoresis; Cinnarizine; Domperidone; Nicergoline; Co-formulated tablets

1. Introduction

Cinnarizine [(1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine] (CN; Fig. 1) is a piperazine derivative with antihistaminic, sedative, and calcium channel-blocking activity. CN is used for the symptomatic treatment of nausea and vertigo caused by Meniere's disease and other vestibular disorders, and it is also used for the prevention and treatment of motion sickness [1,2]. Recently, CN has become readily available as a co-formulated pharmaceutical preparation with domperidone (DOM, 5-chloro-1-[1-[3-(2-oxo-1-benzimidazolinyl) propyl]-4-piperidyl]-2-benzimidazolinone; Fig. 1). Domperidone is a dopamine antagonist used as a prokinetic drug to treat gastrointestinal motility disorders [1,2]. A combined dosage of CN and DOM is more effective for controlling motion sickness compared with the individual use of either drug [3]. Additionally, CN and nicergoline (NIC, 1-methyllumilysergol 8-(5-bromonicotinate) 10-methyl ether; Fig. 1) are also co-formulated in a pharmaceutical preparation for the treatment of cerebrovascular disorders, senile dementia, and memory disorders. The simultaneous dosage of CN and NIC is also effective for the treatment of cerebral decay because NIC improves intellectual, affective, behavioral, and somatic disturbances, whereas CN inhibits vascular smooth muscle cell contractions through calcium channel blocking. Determination of CN/DOM (CN and DOM) and CN/NIC (CN and NIC) in their co-formulated tablets is important for estimating the appropriate dose for the control of clinical symptoms; therefore, the development of a simple, rapid, and sensitive analytical method for the simultaneous determination of CN/DOM and CN/NIC in their co-formulated tablets is important.

Thus far, analytical methods to determine CN, DOM, and NIC in both pharmaceutical preparations and biological fluids have been frequently reported. Four analytical methods – one

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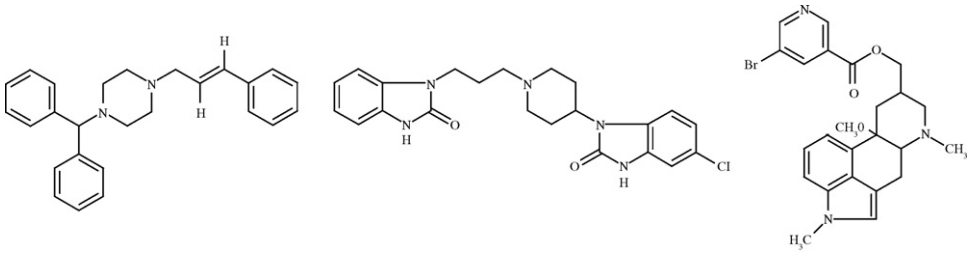

Fig. 1. Structural formulae of the studied drugs.

based on derivative ratio spectrophotometry [4] and three based on high-performance liquid chromatography [5–7] – have also been proposed for the simultaneous determination of CN and DOM. However, HPLC procedures for CN and DOM have a long analysis time of approximately 12 min. Furthermore, thus far, no analytical method has been reported for the simultaneous determination of CN and NIC.

Capillary electrophoresis (CE) has become a useful and powerful separation technique because of its low cost of analysis, small sample volume, minimal running buffer volume, short analysis time, high separation efficiency, and high selectivity. Recently, CE techniques have been applied to the simultaneous determination of co-formulated drugs in dosage form [8–16]. To the best of our knowledge, a CE method for the determination of CN/DOM and CN/NIC has not been reported.

In this study, sensitive, rapid, and accurate CE methods were developed for the determination of CN, DOM, and NIC in mixtures of CN/DOM and CN/NIC. The proposed methods were validated and applied to the simultaneous determination of CN/DOM and CN/NIC in co-formulated preparations.

2. Experimental

2.1. Materials and reagents

Pure compounds of CN, DOM, and NIC were purchased from Sigma (St. Louis, MO, USA). Touristil tablets (labeled to contain 20 mg CN and 15 mg DOM per tablet of ca. 170 mg; batch #020071; product of Janssen Cilag Company, Cairo, Egypt, under license of Janssen Pharmaceutical, Belgium) and cinibral tablets (labeled to contain 25 mg CN and 10 mg NIC per tablet of c.a. 260 mg; batch #0412002; product of Sigma Pharmaceutical Industries, Menofya, Egypt) were used as the co-formulated drugs. Internal standards (IS) were used for better quantitative analysis in order to reduce injection-related imprecision [17] and to ensure reproducibility. To select the IS, quinine HCl (QU), nicardipine HCl (NC), lidocaine, benzocaine, verapamil, diltiazem HCl, and diazepam were tested (all compounds were purchased from Sigma). QU and NC were selected for the determination of CN/DOM and CN/NIC, respectively. Thiourea (TU; Wako Pure Chemical Industries, Osaka, Japan) was used as the electroosmotic flow (EOF) marker. Reagents for the buffer solution (sodium acetate, sodium tetraborate, sodium dihydrogen phosphate dehydrate, boric acid, phosphoric acid, glycine, methanol, acetonitrile, ethanol, and acetic acid) were purchased from Wako.

2.2. Preparation of standard and working solutions

Stock and working standards were prepared as follows. Ten milligrams each of CN, DOM, NIC, TU, and the IS (QU and NC) was separately weighed and dissolved with 100.0 ml of methanol (six stock standard solutions were prepared). Working standards were prepared by further diluting the stock standard solutions with the running buffer for CE separation. The standard solutions were stable for 10 days when maintained in a refrigerator at 4 °C; however, the NIC standard solution was unstable and had to be freshly prepared daily.

2.3. Sample preparation of dosage forms

Twenty composite tablets containing either CN/DOM or CN/NIC were weighed and crushed into a fine powder in a mortar. An exactly weighted portion of the powdered tablets (170 mg touristil tablets (equivalent to 20 mg CN and 15 mg DOM) or 260 mg cinibral tablets (equivalent to 25 mg CN and 10 mg NIC)) was transferred into a 100 ml flask. The contents of the flask were successively extracted with 3 × 30 ml of methanol. The extract was filtered into another 100 ml volumetric flask. The flask used for the extraction was washed with a few milliliters of methanol. The washings were also passed into the volumetric flask; the flask was then filled with methanol up to the mark. Prior to sample analysis, 1 ml of the filtered solution was transferred into a 10 ml volumetric flask and diluted with running buffer before use. For all quantitative determinations, a constant amount (45 μg/ml) of TU (EOF marker) and IS (20 μg/ml) was added to the drug solutions.

2.4. Apparatus and electrophoretic conditions

CE experiments were performed using a CAPI-3100 CE system (Otsuka Electronics, Osaka, Japan) equipped with a multiwavelength UV–VIS detector and an automatic sampler. An uncoated fused-silica capillary of 50 μm i.d. (supplied from GL Science, Tokyo, Japan) with a total length of 40 cm (effective length, 28 cm) was used for the separation. Before use, the new capillary was pretreated by rinsing with 1 M sodium hydroxide for 15 min, water for 10 min, and finally with the buffer solu-

tion for 7 min. Between each run, a vacuum suction was used to sequentially flush the capillary with 0.1 M sodium hydroxide, water, and the buffer solution for 3 min each. All solutions were degassed before use.

The electrophoretic conditions after optimization were as follows: running buffer, methanol–acetate buffer (pH 3.0; 10 mM) (80:20 and 85:15 (v/v) for CN/DOM and CN/NIC, respectively); applied voltage, 20 kV; UV detection wavelength, 215 and 227 nm for CN/DOM and CN/NIC, respectively; hydrodynamic injection was performed at a height of 25 mm for 30 s.

3. Results and discussion

3.1. Optimization of the CE conditions

3.1.1. Organic modifier and concentration effects

The organic modifier in the running buffer (pH 3.0; acetate buffer) plays an important role in electrophoretic separation. Acetonitrile, ethanol, and methanol were tested as organic modifiers (85%, v/v). Methanol gave the best result in terms of resolution, peak shape, and analysis time. The effect of methanol concentration in the range of 10–90% (v/v) was also studied. When the methanol concentration was less than 50% (v/v), no separation in the case of both CN/DOM and CN/NIC was observed. As shown in Fig. 2, although the analysis times were slightly prolonged, for both CN/DOM and CN/NIC, an increase in the methanol concentration resulted in an enhancement in the resolution of the separation. The resolution between CN and DOM was constant at methanol concentrations greater than 80% (v/v) and that between CN and NIC was identical for methanol concentrations of 85 and 90% (v/v). Therefore, 80% (v/v) methanol was selected as the optimum concentration of the organic modifier for CN and DOM, and a concentration of 85% (v/v) methanol was selected for the separation of CN and NIC. The mechanism responsible for the increase in the resolution of separation with an increase in methanol concentration is still under investigation; however, shifts in the pKa values of the analytes, solvation variation, and the interaction between the analytes and buffer reagents may play an important role in this phenomenon. Because the relative standard deviations (R.S.D.) in the resolution for individual methanol concentrations were very small (less than 4% for $n = 3$), the resolution error bar was not displayed in Fig. 2.

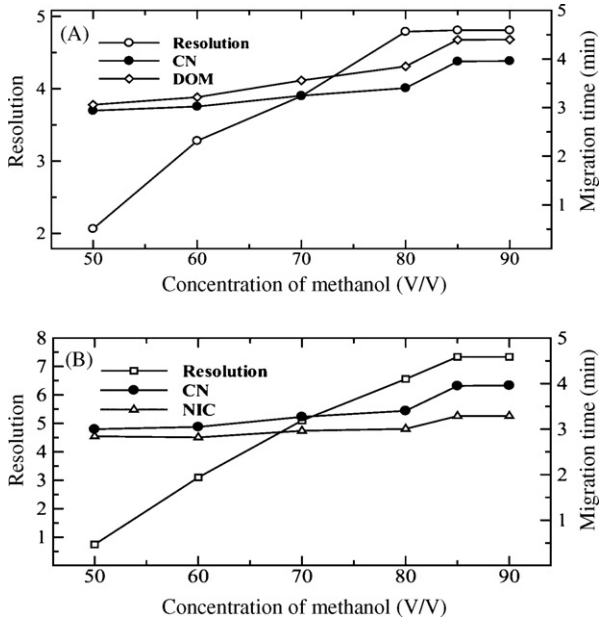

Fig. 2. Effect of methanol concentration on resolution and migration times. (A) CN (20 μg/ml) and DOM (15 μg/ml); (B) CN (25 μg/ml) and NIC (10 μg/ml). Running buffer, 10 mM acetate buffer (pH 3) containing various amounts of methanol; detection at 215 and 227 nm for (A) and (B), respectively; applied voltage, 20 kV; hydrodynamic injection, 25 mm for 30 s.

3.1.2. Buffer pH and type effects

The influence of the pH values ranging from 2.0 to 9.0 of various types of buffer solutions on the separation was investigated because it dominates the EOF velocity and electrophoretic mobility of each analyte due to the magnitude of the ionization. All buffer concentrations were adjusted to 10 mM. Phosphate buffer often leads to the formation of dissolved small particles, which is a source of noises, in the running buffer saturated with organic modifier, and it was considered unsuitable for use in this experiment.

In the pH range of 7.6–9.0 (borate buffer), peaks of CN, DOM, NIC, and EOF markers were not resolved. As shown in Fig. 3, the best separations between CN and DOM or NIC peaks were achieved at pH 3.0 (acetate buffer) along with maximum resolution (>7.3), the best peak shape, and a stable baseline. Separation at pH 2.0 (glycine–HCl buffer) was not as effective as that at pH 3.0 (acetate buffer). Moreover, a glycine–HCl buffer of pH

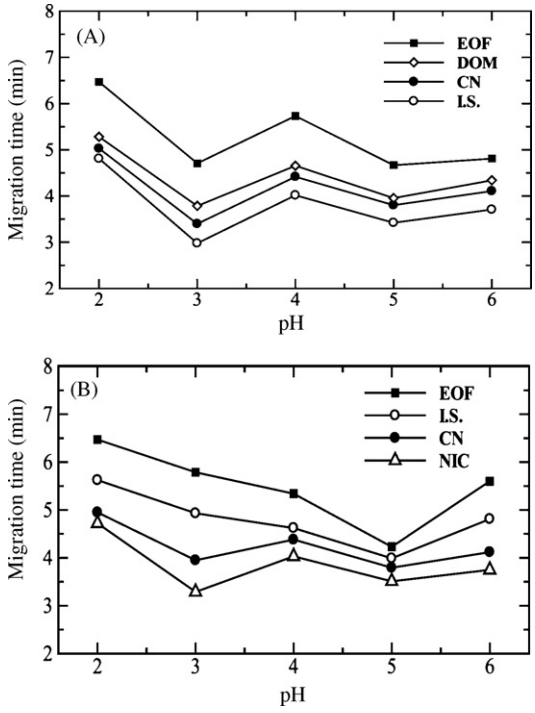

Fig. 3. Effect of pH on migration times of (A) CN/DOM/IS/EOF and (B) CN/NIC/IS/EOF. Buffers of glycine–HCl (pH 2.0) and acetate (pH 3.0–6.0) were used. The other conditions are the same as those in Fig. 2.

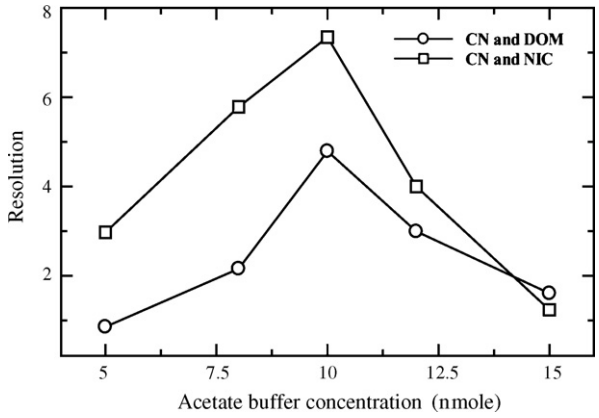

Fig. 4. Effect of acetate buffer (pH 3.0) concentration on the resolution of the separation of CN/DOM and CN/NIC. The other conditions are the same as those in Fig. 2.

3.0 gave lower resolutions compared with an acetate buffer of the same pH. Therefore, an acetate buffer of pH 3.0 was deemed optimal for use throughout the study.

3.1.3. Effect of buffer concentration

The effect of the acetate buffer concentration at pH 3.0 was studied by varying the concentration from 5 to 15 mM. Fig. 4 shows the relationships between the acetate buffer concentration and the observed resolutions for CN/DOM and CN/NIC. Increases in buffer concentration up to 10 mM improved resolution in the case of both CN/DOM and CN/NIC separation. Although the mechanism responsible for this improvement in resolution has not yet been clarified, an increase in the interaction between analyte cations and acetate anions under methanol-rich conditions may explain the increase in the resolution. When the concentration increased beyond 10 mM, unstable baselines and peak broadening were noted. This may be due to elevated temperatures caused by Joule heating due to increase in current density [18]. The best resolutions were thus achieved at 10 mM in the separations of both CN/DOM and CN/NIC. Therefore, a 10 mM acetate buffer was selected for the separations of both CN/DOM and CN/NIC. The R.S.D. of each resolution was within 4%.

3.1.4. Applied voltage effect

Applied voltage significantly affects migration time, current intensity, and resolution. The influence of the applied voltage (from 5 to 30 kV) on the separation was evaluated. Analysis time shortened at higher applied voltages, but the peak shape and resolution decreased due to higher Joule heating [19]. Lower voltages resulted in longer migration times and caused lower resolution. Maximum resolution was obtained at 20 kV for the separations of both CN/DOM and CN/NIC in a 10 mM acetate buffer at pH 3.0; therefore, 20 kV was selected as the optimum applied voltage.

3.1.5. Selection of the detection wavelength

A multiwavelength detection system (190–400 nm) was used in the CE system. For the simultaneous determination of

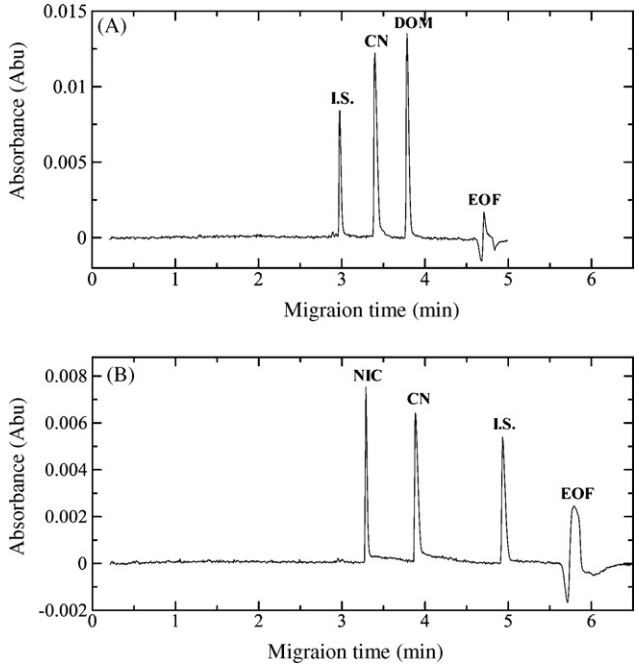

Fig. 5. Electropherograms of standard solutions. (A) CN (20 μg/ml), DOM (15 μg/ml), TU as the EOF marker (45 μg/ml), and IS (QU, 20 μg/ml) and (B) CN (25 μg/ml), NIC (10 μg/ml), TU as the EOF marker, and IS (NC, 20 μg/ml). Running buffer, 10 mM acetate buffer (pH 3) containing (A) 80% and (B) 85% methanol; detection at 215 nm and 227 nm for (A) and (B), respectively; applied voltage, 20 kV; hydrodynamic injection, 25 mm for 30 s.

CN/DOM, the maximum detection sensitivities (the best signal-to-noise ratios) for both CN and DOM were obtained at 215 nm. However, the detection sensitivity of NIC at 215 nm was fairly low. Therefore, a wavelength of 227 nm, which displayed relatively high sensitivity for NIC, was used for the simultaneous determination of CN/NIC, although the sensitivity of CN at 227 nm was slightly lower than that at 215 nm. Detection wavelengths of 215 and 227 nm were selected for CN/DOM and CN/NIC, respectively.

3.1.6. Separation of CN/DOM and CN/NIC under optimized conditions

The separation of working standards under optimized conditions is shown in Fig. 5. Baseline separations of CN/DOM and CN/NIC were achieved within 6 min with high resolutions of 4.80 and 7.30, respectively. The analysis time for CN/DOM was successfully reduced to half of that by the previous HPLC method [6].

3.2. Method validation

3.2.1. Linearity, limit of detection, and limit of quantitation

Calibrations for the simultaneous determination of CN/DOM and CN/NIC were constructed using the relationships between the peak area ratio of sample to IS and sample concentration under optimized CE conditions. The calibration details are listed in Table 1. Good correlation coefficients (>0.9999) were achieved for all compounds. The detection limit (LOD, 3 σ/S_a, where S_a and σ are, the slope and the standard deviation

Table 1
Calibration details

Parameters	CN/DOM		CN/NIC	
	CN	DOM	CN	NIC
Linear concentration range confirmed (μg/ml)	0.25–20	0.375–15	0.25–25	0.4–10
Correlation coefficient	0.9999	0.9999	0.9999	0.9999
Slope ± S.D.	0.1225 ± 0.0001	0.1257 ± 0.0005	0.0517 ± 0.0001	0.0851 ± 0.0001
Intercept ± S.D.	−0.0050 ± 0.0013	0.0041 ± 0.0050	−0.0026 ± 0.0007	0.0006 ± 0.0005
Limit of detection (LOD) (μg/ml)	0.074	0.119	0.072	0.116
Limit of quantitation (LOQ) (μg/ml)	0.247	0.397	0.240	0.386

(S.D.) of the intercept of the regression line of the calibration curve, respectively) and the quantitation limit (LOQ, 10 σ/S_a) were determined according to ICH Q2B recommendations [19]. The detection limits were 0.033/0.119 and 0.041/0.019 μg/ml for CN/DOM and CN/NIC, respectively. In the case of the simultaneous determination of CN and DOM, the linear concentration range of CN in the proposed method was at least 0.25–25 μg/ml.

3.2.2. Precision

The precision of the method was tested with regard to both the intra-day and inter-day reproducibility of the assay. The intra-day variability of the assay was determined by repeated analysis of 3 concentration pairs of CN/DOM and CN/NIC ($n = 7$). Similarly, the inter-day variability of the assay was determined through replicate analysis of 2 concentration pairs of CN/DOM and CN/NIC ($n = 3$), and the results are listed in Table 2. Both the intra- and inter-day reproducibilities of the presented methods were fairly good (%R.S.D. ≤ 1.59). The relative differences between the inter- and intra-day variations for all the analytes were less than 1.13%, and significant precision was obtained.

3.2.3. Robustness of the methods

The robustness of the proposed methods was evaluated by examining the constancy of the peak area ratio between each drug component and IS under the deliberate changes in the experimental parameters (pH, 3 ± 0.5; methanol content, 80 or 85 ± 2.5% (v/v); applied voltage, 20 ± 2 kV). These minor changes from the optimized conditions barely affected the peak area ratio of the studied drugs, and the relative deviations for the peak-to-peak resolutions between CN/DOM and CN/NIC were within ±3 and ±4%, respectively.

3.2.4. Stability of the prepared mixture solutions

The stability of the prepared mixture solutions of the analytes (stock solution diluted with running buffer) was evaluated by CE measurements under optimized conditions. Decreases in the analyte peak areas and the appearance of additional peaks were not observed in the replicate measurements for 10 h. This

Table 2
Intra-assay and inter-day precision of the proposed methods

Mode of precision	Mixture	Drug	Added concentration	*n*	Average	% R.S.D.	%Difference
Intra-day	CN/DOM	CN	20	7	20.10	0.41	0.50
		DOM	15	7	15.09	0.40	0.60
		CN	18	7	18.08	0.33	0.44
		DOM	13.5	7	13.55	0.44	0.38
		CN	16	7	16.09	0.62	0.56
		DOM	12	7	12.04	0.58	0.33
	CN/NIC	CN	25	7	25.06	0.60	0.24
		NIC	10	7	10.04	0.90	0.40
		CN	20	7	20.12	0.65	0.60
		NIC	8	7	7.98	0.50	−0.25
		CN	15	7	15.04	0.66	0.27
		NIC	6	7	6.01	1.00	0.17
Inter-day	CN/DOM	CN	20	3	20.07	0.95	0.35
		DOM	15	3	14.97	1.27	−0.20
		CN	16	3	16.18	1.38	1.13
		DOM	12	3	12.09	1.19	0.75
	CN/NIC	CN	25	3	25.15	0.80	0.60
		NIC	10	3	10.05	1.29	0.50
		CN	20	3	19.99	1.44	−0.05
		NIC	8	3	7.96	1.59	−0.50

Table 3
Determination of CN/DOM and CN/NIC in co-formulated preparations

Preparation	Drug	Labeled claim (mg/tablet)	Amount found (mg/tablet)	Difference (%)	% R.S.D. ($n=3$)	F-test[d]	t-test[d]
Touristil tablets[a]	CN	20	19.73	−1.35	1.62	18.93 (<19.00)	2.754 (<2.776)
	CN[c]	20	20.27	1.35	0.29		
	DOM	15	14.65	−2.33	0.89	4.40 (<19.00)	1.691 (<2.776)
	DOM[c]	15	14.79	−1.40	0.41		
Cinibral tablets[b]	CN	25	25.17	0.68	2.34		
	NIC	10	9.96	−0.40	1.90		

[a] Product of Janssen Cilag Company (Cairo, Egypt) containing 20 mg CN and 15 mg DOM per tablet.
[b] Product of Sigma Pharmaceutical Industries (Menofya, Egypt) containing 25 mg CN and 10 mg NIC per tablet.
[c] Values obtained by derivative ratio spectrophotometry [4].
[d] Values in parentheses are the tabulated t and F at $p=0.05$ [20].

indicates that the prepared mixture solutions of CN/DOM and CN/NIC were stable for at least 10 h.

3.3. Determination of CN/DOM and CN/NIC in co-formulated tablets

The developed CE methods were applied to the simultaneous determination of CN/DOM (touristil tablets, 20 mg CN and 15 mg DOM per tablet) and CN/NIC (cinibral tablets, 25 mg CN and 10 mg NIC per tablet) in their co-formulated tablets. Tablet excipients, such as talc, lactose, starch, avisil, gelatine, and magnesium stearate, present in the matrix did not result in any extra peaks as shown in Fig. 6. Furthermore, the migration times of each analyte shown in Fig. 6 were the same as those in Fig. 5. As listed in Table 3, the amounts of CN/DOM and CN/NIC estimated by the presented methods were almost the same as the certified values on the labels (% difference was within 2.33%) and %R.S.D. values for 3 measurements were less than 2.4%. Comparison between the values obtained by this method and derivative ratio spectrophotometry (a previously reported method) [4] for the simultaneous determination of CN and DOM showed no significant differences with regard to Student's t-test and the variance ratio F-test.

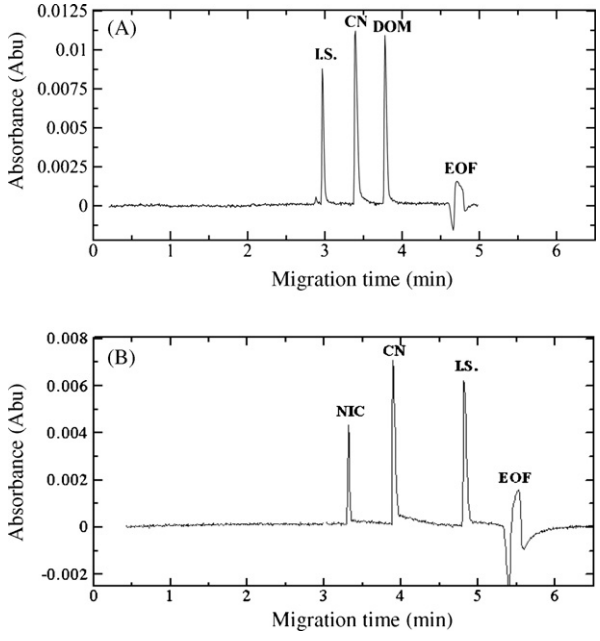

Fig. 6. Electropherograms of (A) CN and DOM in the touristil tablets and (B) CN and NIC in the cinibral tablets with IS (20 μg/ml) and EOF marker (45 μg/ml). The other conditions are the same as those in Fig. 5.

4. Conclusion

Simple and sensitive CE methods were developed for the simultaneous determination of CN/DOM and CN/NIC. Under optimized conditions, baseline separations of CN/DOM and CN/NIC were achieved within 6 min with high resolutions of 4.80 and 7.30, respectively. This analysis time was shorter than that of a previous method, and the determined values showed no statistical difference. The method developed in this study was useful for the determination of CN/DOM and CN/NIC in their co-formulated preparations.

References

[1] S. Budavari, The Merck Index, 12th ed., Merck & Co. Inc., White House Station, NJ, 1996.
[2] K. Parfitt, Martindale: The Complete Drug Reference, 32nd ed., The Pharmaceutical Press, MA, 1999, pp. 411–915.
[3] W.J. Oosterveld, Aviat. Space Environ. Med. 58 (1987) 218–223.
[4] M.Y. Salem, M.G. El-Bardicy, M.F. El-Tarras, E.S. El-Zanfally, J. Pharm. Biomed. Anal. 30 (2002) 21–33.
[5] S.S. Zarapkar, N.P. Bhandari, U.P. Halker, Indian Drugs 37 (2000) 295–298.
[6] A.P. Argekar, S.J. Shah, J. Pharm. Biomed. Anal. 19 (1999) 813–817.
[7] A.P. Argekar, S.G. Powar, J. Planar-Chromatogr. Mod. TLC 12 (1999) 272–274.
[8] P. Srisom, B. Liawruangrath, S. Liawruangrath, J.M. Slater, S. Wangkarn, J. Pharm. Biomed. Anal. 43 (2007) 1013–1018.
[9] P.P. Maia, J. Amaya-Farfán, S. Rath, F.G.R. Reyes, J. Pharm. Biomed. Anal. 43 (2007) 450–456.
[10] R. Hamoudova, M. Pospisilova, J. Pharm. Biomed. Anal. 41 (2006) 1463–1467.
[11] S. Weia, H. Guo, J.M. Lin, J. Chromatogr. B 832 (2006) 90–96.
[12] A. Ahmed, H.A. Hamed, M.I. Abubakr, Talanta 72 (2007) 842–846.
[13] M.E. Capella-Peiró, D. Bose, M.F. Rubert, J. Esteve-Romeroc, J. Chromatogr. B 839 (2006) 95–101.
[14] M.E. Capella-Peiró, A. Bossi, J. Esteve-Romero, Anal. Biochem. 352 (2006) 41–49.
[15] M.I. Bailon Perez, L. Cuadros Rodrıguez, C. Cruces-Blanco, J. Pharm. Biomed. Anal. 43 (2007) 746–752.
[16] A. Maciàa, F. Borrulla, M. Calulla, C. Aguilar, Trend Anal. Chem. 26 (2007) 133–153.

[17] K.D. Altria, H. Fabre, Chromatographia 40 (1995) 313–320.
[18] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, NY, 1993.
[19] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration (2001) Center for Drug Evaluation and Research, Rockville, MD, http://www.fda.gov/eder/guidance/4252fnl.pdf (accessed September 1, 2004).
[20] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed., Wiley, NY, 1993, p. 115.