A capillary zone electrophoresis (CZE) method for the determination of cyclizine hydrochloride in tablets and suppositories

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Abstract

Current compendial methods of assay for the analysis of cyclizine tablets involve the use of UV spectrophotometry. Since this is a non-selective technique its application to more complex dosage forms, such as suppositories, is unlikely to be appropriate. There is therefore a need for the development of a highly specific quantitative analytical method, such as high performance liquid chromatography (HPLC) or capillary electrophoresis (CE). The latter technique was chosen in view of some specific advantages over HPLC, such as the use of relatively non-toxic aqueous buffers, as opposed to organic solvents, which obviates the use of expensive HPLC grade solvents making CE more cost effective. Cyclizine was analyzed in 50 mM phosphate buffer (pH 2.3) and run at an applied voltage 25 kV. Detection sensitivity was enhanced by using a wavelength of 200 nm and samples were loaded hydrodynamically onto an uncoated fused-silica capillary (60 cm×50 mm i.d.). Chlorcyclizine was used as the internal standard and resolution of both compounds was achieved in less than 7 min. Stress testing was undertaken in order to investigate the appearance of breakdown products. The method has the requisite accuracy, selectivity, sensitivity and precision to assay cyclizine in tablets and suppositories. Degradation products resulting from the stress studies did not interfere with the detection of cyclizine and the assay is thus stability-indicating.

1. Introduction

Cyclizine, 1-(diphenylmethyl)-4-methylpiperazine or 1-benzhydryl-4-methylpiperazine [1], is a piperazine derivative that has been used effectively for the prevention and treatment of nausea and vomiting associated with motion sickness [2 and 3].

Cyclizine hydrochloride can be administered orally (50 mg) and/or rectally (50 or 100 mg) three times a day as the recommended dosage regimen. Compendial methods [5 and 6] for the assay of cyclizine in tablets use a non-selective UV spectrophotometric method. Published methods for the determination of

cyclizine in dosage forms include colorimetry [7], potentiometry [8], second derivative UV spectrophotometry [9] and HPLC [10, 11 and 12].

Capillary zone electrophoresis (CZE) is gradually gaining acceptance as an alternative and complementary technique to high performance liquid chromatography (HPLC) for the analysis of pharmaceutical dosage forms [13]. Several recent publications have described the utility of capillary electrophoresis (CE) for the determination of medicinal compounds in dosage forms [14, 15, 16, 17 and 18] and for raw material and related substances [19]. Principle advantages of CZE include, amongst others, high separation efficiency, improved selectivity, low operational costs and speed of analysis [20 and 21]. Specific requirements for the validation of CE methods have been published [22 and 23].

2. Experimental

2.1. Reagents and chemicals

Sodium hydroxide pellets (Rochelle Chemicals, Port Elizabeth, South Africa), hydrogen peroxide solution (30 vol.) (Riedel-de-Haen, Sigma-Aldrich (Pty) Ltd, Johannesburg, South Africa), 85% (v/v) ortho-phosphoric acid (Merck, Midrand, South Africa) and hydrochloric acid (concentrated) (Merck KGaA, Darmstadt, Germany) were of analytical reagent grade and were used without additional purification.

Cyclizine hydrochloride BP (CYC) was obtained from Lennon Ltd Laboratories, South Africa, and the internal standard, chlorcyclizine hydrochloride (98.8%) (CLCYC), was obtained from the Wellcome Foundation Ltd, UK.

Polished HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

Valoid® (GlaxoWellcome, South Africa) tablets and suppositories each containing a label claim of 50 mg cyclizine hydrochloride, which corresponds to 43.989 mg of cyclizine free base, were purchased from a local pharmacy.

2.2. Equipment and capillary electrophoretic conditions

CZE determinations were performed on a Prince CE System, (Prince Technologies, Emmen, The Netherlands).

Analysis was performed in an uncoated fused-silica capillary of total length 60 cm and effective length 43 cm×50 μm i.d.×363 μm o.d. (Polymicro Technologies, Phoenix, AZ, USA). A short capillary with a small diameter was used in order to avoid siphoning effects and analytical difficulties associated with Joule heating. Separations were performed at ambient temperature and detection was by UV absorption at 200 nm using a Linear UVis Model 200 ultraviolet detector (Linear Instruments Corp., Reno, NV, USA). A Millenium® (Waters Chromatography Division, Milford, MA, USA) Chromatographic Data System was coupled to the detector via a SAT/IN Module (Waters Chromatography Division) and used to record and evaluate the data.

A Crison® Model GLP 21 pH meter (Barcelona, Spain) and a Crison® Combination Electrode (ICR151-23) was used to adjust the pH of the electrolyte solutions and a Branson B-12 (Branson Cleaning Equipment Company, Shelton, CT, USA) sonicator facilitated sample solution.

Preliminary studies were performed over the pH range 2–5 using buffer concentrations of 50 and 100 mM. Samples were injected hydrodynamically at 50 mbar for 10 s. The background electrolyte was 50 mM phosphate buffer at pH 2.3 and a constant voltage of 25 kV was applied to obtain the separation. Typical current levels for this separation were of the order of 36 μA throughout the runs.

Before use, each new capillary was washed with 1.0 M sodium hydroxide for 1 h, 0.1 M sodium hydroxide for 1 h, deionized water for 1 h and finally with CZE buffer for 45 min. After each run the capillary was rinsed with run buffer for 5 min. For storage overnight, the capillary was washed with deionized water for 1 h, 0.1 M sodium hydroxide for 1 h and finally with water for a further hour.

2.3. Standards and sample solutions

2.3.1. Standard stock solutions

A standard stock solution of cyclizine (1 mg/ml) and the internal standard (1 mg/ml) were prepared in water. These solutions were protected from light using foil and stored at 4 °C for 10 days and were found to be stable during this period.

2.3.2. Standard solutions for CZE

Aliquots of standard stock solution of cyclizine were dispensed into 10 ml volumetric flasks containing 200 μl of IS solution, and the flasks made up to volume with 10 mM phosphate buffer (pH 2.65), to give final concentrations of 1, 2, 5, 10, 25 and 50 μg/ml. The buffer solution was made up by diluting 20 ml of sodium phosphate buffer (50 mM, pH 2.3) to 100 ml. The final concentration of IS was 20 μg/ml.

2.4. Background electrolyte (BGE)

The running buffer or BGE was made by addition of 3.4 ml of phosphoric acid (85%, w/v) into a 1000 ml volumetric flask and dilution to a constant volume with water. The pH was adjusted to 2.3 with sodium hydroxide and all solutions were freshly prepared and filtered using a Durapore® 0.45 μm HVLP membrane (Millipore) each day.

2.5. Preparation of tablets for assay

Thirty tablets were weighed and powdered. An amount of powder equivalent to the weight of one tablet was dissolved in water in a 50 ml volumetric flask. The flasks were sonicated for 30 min to effect complete dissolution. Suitable aliquots of solution were filtered through a Millex®-HV hydrophilic PVDF 0.45 μm syringe filter (Millipore). A 200 μl aliquot of the filtered solution was added to a 10 ml volumetric flask containing 200 μl IS solution and made up to volume with 10 mM phosphate buffer prepared as previously described.

2.6. Preparation of suppositories for assay

Eight suppositories were accurately weighed into a beaker and melted in a water bath at 55 °C. The melt was thoroughly mixed with a magnetic stirrer. The molten mix was placed in a refrigerator and allowed to solidify. After solidification the solidified mass was broken up and a portion equivalent to the weight of one suppository was quantitatively transferred into a beaker. The beaker was again placed into a water bath at 55 °C in order to melt the mass. Water (15 ml) heated to 55 °C was added to the melt and mixed with a magnetic stirrer for 15 min. The molten mix was then cooled in a refrigerator for 15 min and following solidification of the lipophilic phase, the aqueous phase was decanted and filtered through ashless filter paper (Dassel-W, Germany) into a 50 ml volumetric flask. After filtration, the filter paper was returned to the beaker and the process repeated two more times under the same conditions using a new filter paper each time. Finally, the filter papers were washed with an appropriate amount of hot water, cooled and added to the volumetric flask containing the aqueous phase and made up to volume with water. A portion of this solution was filtered through a Millex®-HV filter and a 200 µl aliquot of the filtered solution was added to a 10 ml volumetric flask containing 200 µl IS solution and made up to volume with 10 mM phosphate buffer prepared as previously described.

2.7. Stress testing of tablets and powder (forced degradation studies)

In order to ensure that the assay is stability-indicating, cyclizine tablets and cyclizine active pharmaceutical ingredient (API) powder were stressed under various conditions in order to force degradation. All solutions were prepared to yield starting concentrations of 20 µg/ml.

2.7.1. Photostability

About 30 mg of API powder was spread in a layer of less than 1 mm on a glass dish. Solutions of the API (1 mg/ml) were prepared in water. Tablets were prepared for exposure in the same way. All samples were placed in a cabinet (Suntest CPS/CPS^{+}, Atlas Material Testing Technology, Germany) and exposed to light for 40 h resulting in an overall illumination of $\geq 1.68 \times 10^{6}$ lux hours at 35 °C. Concurrently, control samples which consisted of all preparations previously described but protected with aluminium foil were also placed in the light cabinet. Following removal from the light cabinet, all samples for analysis were prepared as previously described.

2.7.2. Oxidation

Solutions for oxidation studies were prepared in hydrogen peroxide (10%, v/v), protected from light and stored at room temperature for 5 days. API (1 mg/ml) and tablet powder equivalent to 50 mg cyclizine hydrochloride were prepared as previously described.

2.7.3. Acid degradation studies

Solutions for acid degradation studies were prepared in 2 M hydrochloric acid, protected from light and stored at room temperature for 5 days. API (1 mg/ml) and tablet powder equivalent to 50 mg cyclizine hydrochloride were prepared as previously described.

2.7.4. Alkali degradation studies

Cyclizine hydrochloride is relatively insoluble in concentrated alkaline solutions. The acid–base protonation constants (pK_a) of cyclizine and chlorcyclizine in aqueous methanol solution are shown in Fig. 1. The specific acid–base behavior of these compounds involves the protonation of the nitrogen located in the piperazine ring. In aqueous-acidic solutions the cationic mono-protonated species will more than likely dominate and have a better solubility. Consequently, a dilute sodium hydroxide solution (0.02 M) was used to expose the drug to alkaline conditions. These solutions were protected from light and stored at room temperature for 5 days.

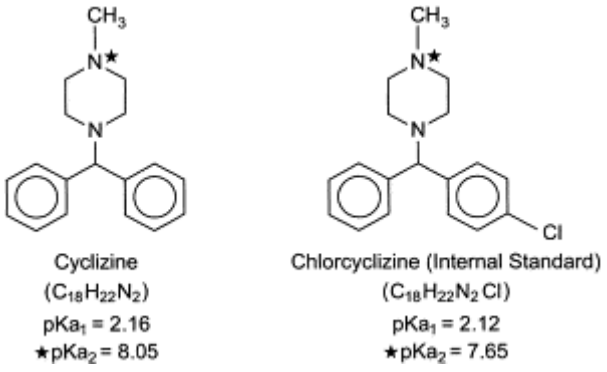

Fig. 1. Structural formulae of cyclizine and chlorcyclizine showing reported pK_a values [**4**].

2.7.5. Temperature stress studies

Tablets were exposed to dry heat (80 °C) in an oven for 4 days. Tablets were removed, powdered and prepared for analysis as previously described.

Solutions prepared for all stress studies were stored at room temperature for 5 days and, where appropriate were protected from light.

3. Results and discussion

3.1. Validation

3.1.1. Linearity

Calibration curves constructed for cyclizine were linear over the entire range from 1 to 50 μg/ml. Peak area ratios CYC/IS were plotted versus cyclizine concentration and linear regression analysis resulted in a correlation coefficient of R=0.9999 with %R.S.D. values ranging from 0.38 to 2.64 across the concentrations studied. Typically, the regression equation for the calibration curve was determined to be y=0.44x+0.0034, where y=ratio of peak area (CYC/IS) and x is the ratio of concentration (CYC/IS) and the intercept, 0.0034, is close to the origin.

3.1.2. Limit of quantification (LOQ) and limit of detection (LOD)

The LOD was distinguished from a signal-to-noise ratio of 3:1 and was determined to be 0.1 μg/ml. The LOQ of 1 μg/ml was established with a %R.S.D. of 2.29%, which is lower than the normal acceptance criteria of 10% [23].

3.1.3. Precision

The assay was investigated with respect to repeatability and inter-day precision. Repeatability was investigated by injecting nine replicate injections of each of 1, 10 and 50 μg/ml standards where the mean

concentrations were found to be 1.02, 10.1 and 50.16 μg/ml with associated %R.S.D.'s of 2.30, 1.22 and 0.38, respectively. The mean migration times for cyclizine and chlorcyclizine were 4.9 and 5.5 min with %R.S.D. of 0.55 and 0.91, respectively.

Inter-day precision was assessed by injecting the same three concentrations over 3 consecutive days, resulting in mean concentrations of cyclizine of 1.00, 10.12 and 50.72 μg/ml and associated %R.S.D. of 3.32, 3.61 and 2.76, respectively.

In addition, two separate capillaries were used and the relevant data are listed in Table 1. It is apparent that the method is reproducible as indicated by calibration and %R.S.D. data presented here.

Table 1. Inter-day precision and ruggedness testing

Cyclizine (μg/ml)	Capillary 1				Capillary 2	
	Day 1 (n = 9)		Day 2 (n = 9)		Day 3 (n = 9)	
	Peak area CYC/IS	%R.S.D.	Peak area CYC/IS	%R.S.D.	Peak area CYC/IS	%R.S.D.
1	0.0472	3.67	0.0484	2.29	0.0466	3.65
2	0.0889	1.53	0.0909	1.97	0.0935	2.27
5	0.2149	1.72	0.2275	2.64	0.2306	2.53
10	0.4310	1.50	0.4478	1.22	0.4671	0.55
25	1.0909	1.87	1.0855	0.84	1.1833	1.12
50	2.2429	0.24	2.2086	0.38	2.3477	0.18

3.1.4. Accuracy

Accuracy of the assay was determined by interpolation of replicate (n=6) peak area ratios of three accuracy standards (1, 10 and 50 μg/ml) from a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy was calculated. The resultant concentrations were 1.03±0.025 μg/ml, 10.11±0.09 μg/ml and 50.06±0.16 μg/ml with percent relevant errors of 3.2, 1.1 and 0.12, respectively.

3.1.5. Selectivity

The results of stress testing studies indicated a high degree of selectivity. Fig. 2 depicts a resultant electropherogram following storage of CYC tablets in water, where degradation was significant. The degradation behavior of cyclizine was similar in both tablets and API powder.

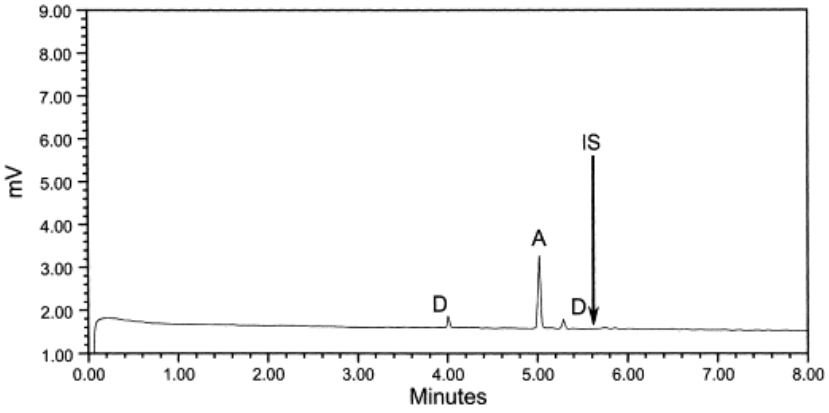

Fig. 2. Resultant electropherogram following storage of cyclizine tablets in water. A, cyclizine; D, unknown degradation products. IS indicates the migration time for the internal standard, chlorcyclizine.

Following photolytic exposure of cyclizine API and tablets in water, a golden yellow solution resulted when compared to the colorless control solution. Exposure of cyclizine API and tablets to hydrogen peroxide yielded solutions that were pale yellow and all other solutions remained colorless. Powdered cyclizine tablets in sodium hydroxide solutions were dark yellow during preparation at room temperature. It is clearly evident that there is no interference of any degradation products with the internal standard.

Cyclizine was found to be stable under heat and acidic stress conditions, whereas under alkali and oxidation conditions degradation occurred and other degradation products were formed only during cyclizine oxidation studies. Five days after the exposure of solutions of cyclizine to 10% (v/v) hydrogen peroxide at room temperature, a small peak eluted at the same migration time for chlorcyclizine (Fig. 3). This peak corresponds to less than 5% degradation and as such was not considered likely to cause interference with the assay. However, as a precaution, extracts of cyclizine product(s) without the addition of internal standard should be run prior to assay in order to establish the presence or absence of any possible interference with the internal standard.

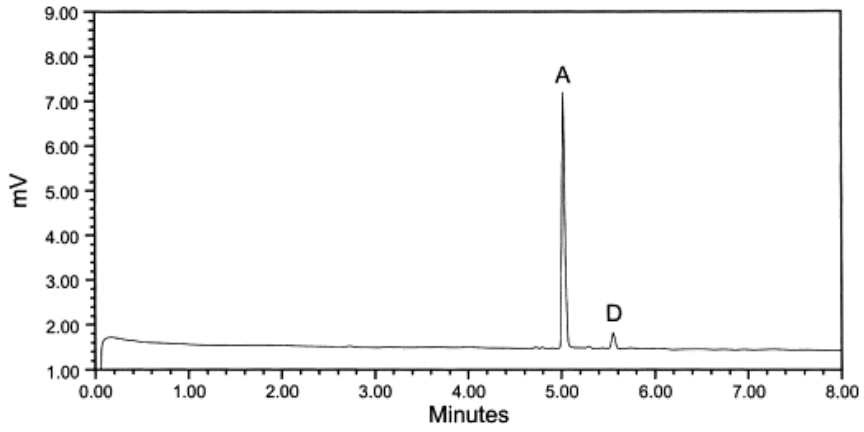

Fig. 3. Resultant electropherogram following storage of cyclizine API in 10% (v/v) hydrogen peroxide for 5 days at room temperature. A, cyclizine; D, unknown degradation product.

3.1.6. Recovery

Known amounts of cyclizine hydrochloride were added to samples of tablets and suppositories, which were then extracted, diluted and analyzed. The final nominal concentrations of cyclizine were 29.12 and 26.15 μg/ml for the tablets and suppositories, respectively. These assays were repeated (n=9) over 5 days to obtain intermediate precision data. The resultant %R.S.D. for these studies were 1.17 and 2.20 with corresponding percentage recovery values of 97.7 and 95.0% for the tablets and suppositories, respectively.

3.1.7. Assay

Typical electropherograms obtained following the assays of both the tablets and suppositories are depicted in Fig. 4. Results of these assays yielded 99.14% (%R.S.D.=1.80) and 99.12% (%R.S.D.=2.60) of label claim for the tablets and suppositories, respectively.

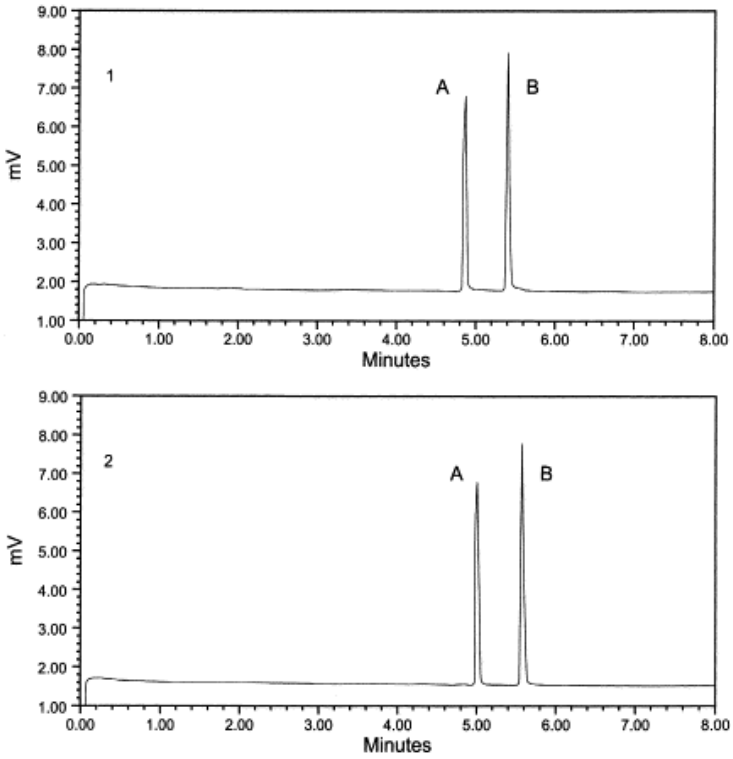

Fig. 4. Resultant electropherograms following the analysis of Valoid® tablets (1) and pediatric suppositories (2) showing cyclizine, A, and chlorcyclizine, B.

4. Conclusion

The reported method has the necessary precision, accuracy, sensitivity and selectivity for the analysis of cyclizine hydrochloride in tablets and suppositories. However, cyclizine products should be run without internal standard during shelf life testing, to preclude potential interference from an oxidative degradation product that has a similar migration time to chlorcyclizine. In this study, cyclizine tablets with an expiry date of October 1993 were also tested and revealed no evidence of oxidative degradation. Therefore, during routine analysis and shelf life testing of products, in which a quick turnaround time is desired, oxidative degradation is unlikely to occur, and thus this method is considered to be stability-indicating.

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