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Method development and validation for the simultaneous determination of cetirizine dihydrochloride, paracetamol, and phenylpropanolamine hydrochloride in tablets by capillary zone electrophoresis☆

S. Azhagvuel, R. Sekar*

Analytical Chemistry Division, Indian Institute of Chemical Technology, Hyderabad 500007, India

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Abstract

A simple, selective, and cost effective capillary zone electrophoresis (CZE) method has been developed for the simultaneous separation and determination of cetirizine dihydrochloride (CTZ), paracetamol (PARA), and phenylpropanolamine hydrochloride (PPA) in tablets. A 10 mM sodium tetraborate background electrolyte (BGE) solution (pH 9.0) was found to be suitable for separation of all the analytes. An uncoated fused-silica capillary of a total length of 76 cm (effective length 64.5 cm) was used for separation. All the analytes were completely separated within 10 min at the applied voltage of 20 kV (current produced ~21 μA), and detection was performed at 195 nm with an UV detector. Ibuprofen was used as internal standard (I.S.) for the quantification of the drugs. Validation of the method was performed in terms of linearity, accuracy, precision, limit of detection (LOD), and quantification (LOQ). The linearity of the calibration curves for CTZ, PARA, and PPA (tested range) were 2–50 μg ml^{-1} (r^2 = 0.9982), 10–1000 μg ml^{-1} (r^2 = 0.9978), and 10–100 μg ml^{-1} (r^2 = 0.9986), respectively. The proposed method has been applied for the determination of active ingredients in tablets, and the recovery was found to be ≥98.60% with the relative standard deviation (R.S.D.) ≤1.56%. The LOQ of the CTZ, PARA, and PPA was found to be 2.0, 2.0, and 4.0 μg ml^{-1}, respectively. There were no interfering peaks due to the excipients present in the pharmaceutical tablets. Thus, the proposed method is simple and suitable for the simultaneous analysis of active ingredients in tablet dosage forms.
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Keywords: Capillary zone electrophoresis; Cetirizine dihydrochloride; Paracetamol; Phenyl propanolamine hydrochloride; Pharmaceuticals

1. Introduction

Cetirizine dihydrochloride (CTZ) is [(RS)-2-[2-[4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy] acetic acid dihydrochloride] a non-sedative histamine H1-receptor antagonist used for the treatment of seasonal rhinitis and chronic urtiacaria or pruritsis of allergic origin [1]. Paracetamol (PARA) [*N*-acetyl-p-aminophenol] is used as an analgesic and antipyretic agent [2]. Phenylpropanolamine hydrochloride (PPA) [(1RS,2SR)-2-amino-1-phenylpropanol hydrochloride] is a sympathomimetic compound, which has been widely used as an OTC and prescription medication for cough, cold, and nasal decongestant. The combined dosage form of CTZ, PARA, and PPA is more effective in controlling common cold and severe allergic cases than individual drugs [3].

Literature survey reveals that many methods have been reported for the determination of CTZ [4–13], PARA [14–18], and PPA [19–21] in pharmaceutical formulations either alone or in combination of any two of these three drugs or in combination with other drugs. Spectrophotometric methods [6,7,12,13] usually require sample pre-treatments (extraction, complex formation, derivatization, etc.). High performance liquid chromatography (HPLC) methods have been more frequently used, in which these basic drugs strongly interact with stationary phase of the column causing peak asymmetry and low separation efficiency [22]. There is only one report [3] available in the literature for the simultaneous determination of CTZ, PARA, and PPA using reverse phase high performance liquid chromatography (RP-HPLC). In this method, a high concentration of PARA with the low concentrations of the CTZ and PPA was analyzed by using different wavelengths for quantification. Eventhough different

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* Corresponding author. Tel.: +91 40 27160123; fax: +91 40 27160387.
E-mail address: seker@rediffmail.com (R. Sekar).

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wavelength selection provides sensitive detection of compounds in multi drug dosage form, a significant amount of noise in the calibration is observed thereby decreasing the precision [23].

Capillary electrophoresis (CE) offers possible advantages over HPLC in terms of minimal buffer (mobile phase) and sample volume, high separation efficiency, short analysis time, eco-friendly solvents, and high selectivity. It has been reported as a powerful tool for a wide range of analysis, including many applications to the determination of drugs, drug related impurities determination, and in chiral separations [24]. CE methods [22,25–28] are available for the determination of PARA and PPA drugs alone, or mixture with some other combination dosage forms. To the best of our knowledge, no CE method is available in literature for the determination of CTZ either alone and for simultaneous determination of CTZ, PARA, and PPA in tablet dosage forms.

Hence the present work describes a simple and cost effective capillary zone electrophoresis (CZE) method for the determination of CTZ alone and its combination with PARA, and PPA drugs in multicomponent dosage form. The effects of different buffer, pH, concentration, and applied voltage were studied to optimize the experimental condition. Under the optimized conditions, linearity, precision, accuracy, limit of detection (LOD), and quantification (LOQ) were validated.

2. Experimental

2.1. Instrumentation

CE experiments were performed using a Prince CE system (Prince Technologies, Model No. 460, The Netherlands) equipped with a Lambda 1010 UV–Vis detector, an auto sampler. An uncoated fused-silica capillary of 75 μm i.d. (Polymicro, Phoenix, AZ, USA) with a total length of 76 cm (64.5 cm effective length) was used for the separation. The capillary was thermostated at 25 °C. Samples were kept in the auto sampler and injected by applying a pressure of 50 mbar for 12 s. A constant voltage of +20 kV was applied throughout the analysis. Detections were performed at 195 nm. Data acquisition and analysis were carried out with the DAx software supplied by the Prince Technologies.

A new capillary was conditioned by rinsing with 1.0 M sodium hydroxide for 20 min, water for 10 min, and finally with the buffer solution for 10 min. Between each run, the capillary was rinsed with water for 2 min, 0.1 M sodium hydroxide for 2 min, water for 3 min, and the buffer solution for 3 min successively.

2.2. Chemicals and reagents

All chemicals used in the analysis were of analytical-reagent (AR) grade. Cetirizine dihydrochloride, paracetamol, phenylpropanolamine hydrochloride, and ibuprofen (IBU) pure standard drugs were kindly supplied by Rajendra Pharmaceuticals Ltd., Hyderabad, India. Cetirizine and its combination tablets of different brands were purchased from local pharmacy. Hydrochloric acid, and HPLC grade methanol were purchased from Qualigens Fine Chemicals, Mumbai, India. Sodium dihydrogen phosphate dihydrate, sodium tetraborate decahydrate, and sodium hydroxide were purchased from S.D. Fine Chem. Ltd., Mumbai, India. Deionized water was prepared by using a Milli-Q water purification system Millipore, Molsheim, France.

2.3. Preparation of background electrolyte (BGE) solution and drug standards

The background electrolyte was prepared by dissolving an appropriate amount of sodium tetraborate decahydrate in deionized water. The BGE solution was adjusted to required pH by the addition of 0.1 M sodium hydroxide or 0.1 M HCl and the solution was filtered using 0.45 μm micro syringe filters before analysis.

A stock and working standard of drugs and internal standard were prepared as follows: a 100 mg of each of CTZ, PARA, PPA, and ibuprofen (I.S.) drug standards were separately weighed and dissolved with 100 ml of methanol (1.0 mg ml^{-1}). A required concentration of working standard of individual drugs and mixture (2–1000 μg ml^{-1}) were prepared by diluting the stock standard solutions with deionized water before CE injection.

2.4. Preparation of pharmaceutical samples

2.4.1. Preparation of CTZ tablet sample

Twenty tablets were weighed, crushed into a fine powder in a mortar and homogenized. A portion of the powder equivalent to 10 mg of CTZ was transferred into a 10 ml volumetric flask; 5 ml methanol was added and shaken thoroughly to dissolve, brought the volume to the mark with methanol and sonicated for 15 min. An appropriate volume of filtrated solution was diluted with deionized water to get concentration of 50 μg ml^{-1} CTZ.

2.4.2. Preparation of CTZ composite tablet sample

Twenty composite tablets containing CTZ, PARA, and PPA were weighed, crushed into fine powder in a mortar and homogenized. A portion of the powder equivalent to 5 mg of CTZ, 500 mg of PARA, and 25 mg of PPA was transferred into a 100 ml volumetric flask, dissolved with methanol, and made up to the mark with the same solvent and sonicated for 15 min. Prior to sample analysis, a portion of filtrate solution was diluted with deionized water to get the concentrations of CTZ 4 μg ml^{-1}, PARA 400 μg ml^{-1}, and PPA 20 μg ml^{-1}. For all quantitative determinations, a constant amount of ibuprofen (I.S.) 25 μg ml^{-1} was added to the drug solution.

Sample and standard solutions were stored in a refrigerator at 4 °C. They were stable for the period of one month under these storage conditions. BGE solution and samples were filtered through 0.45 μm micro syringe filters prior to use.

3. Results and discussion

3.1. Optimization of the CE conditions

3.1.1. Buffer selection

In order to obtain the optimum capillary electrophoretic condition, three buffer systems such as sodium hydrogen phosphate,

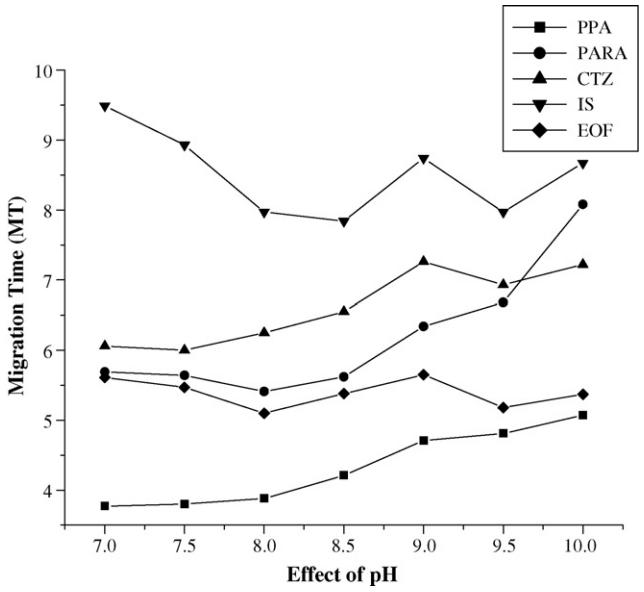

Fig. 1. Effect of pH on migration time of CTZ, PARA, PPA, and EOF with I.S. Electrophoretic conditions: 10 mM sodium tetraborate, hydrodynamic injection (50 mbar for 12 s), applied voltage 20 kV, 25 °C.

sodium tetraborate and sodium phosphate–borate buffer in the pH range 4–10 were investigated. Among these buffers best results were obtained with sodium tetraborate in terms of peak shape, resolution (>3), selectivity and sensitivity (<1 μg ml^{-1}).

3.1.2. Effect of buffer pH

Buffer pH determines the extent of the ionization of each analyte and magnitude of the electroosmatic flow (EOF). To study the effect of pH on the separation of these drugs, a constant concentration of 10 mM sodium tetraborate solution was adjusted to pH range 4–10 and evaluated as background electrolyte. Although the separation of analytes were achieved by using a pH range 7.0–8.5, PARA migrated closely with the EOF (Fig. 1), due to its uncharged nature [29]. At pH 9.5 CTZ and PARA peaks migrated closely, whereas on reaching pH 10.0 CTZ and PARA separation order reversed, but the EOF co-migrated with the PPA. Therefore, finally pH 9.0 was selected for separation of the analytes.

3.1.3. Effect of buffer concentration

The effect of BGE solution concentration was studied by varying sodium tetraborate concentration from 5 to 20 mM at a constant pH of 9.0. When increasing BGE concentration, the peak efficiency and separation of peaks were increased with the increase in the analysis time (Fig. 2). While increasing buffer concentration above 10 mM higher current and baseline shift was observed. Therefore, 10 mM borate buffer concentration was kept constant for the separation of analytes.

3.1.4. Effect of voltage and injection time

The influence of voltage from 5 to 30 kV was evaluated under the optimized BGE conditions. When the applied voltage was greater than 20 kV, peak broadening occurs due to the high

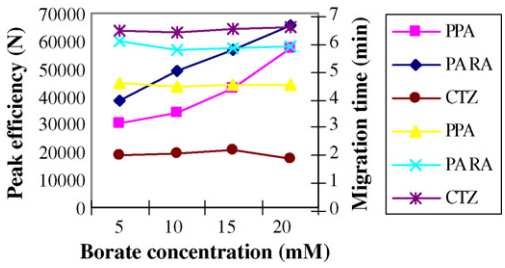

Fig. 2. Effect of buffer concentration on peak efficiency and migration times of drugs. Electrophoretic conditions: 10 mM sodium tetraborate (pH 9.0), hydrodynamic injection (50 mbar for 12 s) applied voltage 20 kV, 25 °C.

currents. The separation voltage of 20 kV produces low current (∼21 μA), high peak efficiency (theoretical plates >20,000) and shorter analysis time (<10 min) Therefore, 20 kV voltage was chosen for separation of the analytes.

Sample injection time (6–12 s) and pressure (10–50 mbar) were varied to achieve a lower detection limit without affecting the quality of the peak shape and reproducibility. An injection pressure of 50 mbar and duration of 12 s offered best results and was selected as optimized injection time and pressure for sample injection.

3.1.5. Selection of detection wavelength

All the analytes have sufficient UV absorption at wavelength 195 nm. Therefore, 195 nm was selected as optimum detection wavelength for simultaneous determination of drugs.

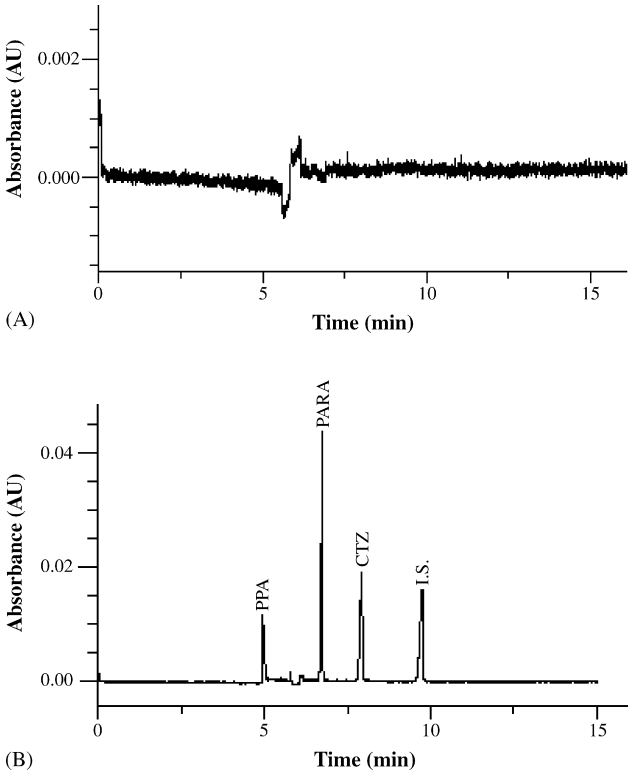

Fig. 3. Electropherograms (A) excipients (placebo) (B) standard solutions of CTZ, PARA, and PPA (50 μg ml^{-1}) with I.S. (25 μg ml^{-1}) spiked in excipients solution. Electrophoretic conditions as given in Fig. 2.

Table 1
Repeatability and intermediate precision data for the drugs PPA, PARA, and CTZ in standard mixtures with respect to peak MT and area

Compounds	Concentration added (μg ml^{-1})	Intra day ($n=5$)		Inter day ($n=7$)	
		MT R.S.D. (%)	Peak area R.S.D. (%)	MT R.S.D. (%)	Peak area R.S.D. (%)
PPA	10	0.26	1.46	1.96	2.92
	20	0.20	1.21	1.73	3.13
	30	0.18	1.28	2.12	3.72
PARA	200	0.22	0.38	1.12	3.18
	400	0.17	0.30	0.99	4.86
	600	0.26	0.42	1.85	4.92
CTZ	2	0.22	0.42	0.86	3.20
	4	0.19	0.35	0.67	4.57
	6	0.14	0.56	0.22	2.87

Table 2
Accuracy and precision data for the assay of PPA, PARA, and CTZ in drug mixtures

Compounds	Concentration added (μg ml^{-1})	Drug substance ($n=5$)		Drug product ($n=5$)	
		Recovery (%)	Repeatability R.S.D. (%)	Recovery (%)	Repeatability R.S.D. (%)
PPA	10	99.22	1.46	99.42	1.52
	20	101.14	1.21	100.35	1.20
	30	100.74	1.28	98.70	1.12
PARA	200	101.68	0.38	100.62	0.52
	400	99.52	0.30	98.86	0.42
	600	99.20	0.42	99.28	0.38
CTZ	2	99.85	0.42	99.56	0.38
	4	99.92	0.35	100.02	0.52
	6	100.16	0.56	99.24	0.42

3.2. Method validation

The analytical method was validated according the International Conference for Harmonization (ICH) guidelines [30] under the optimized experimental conditions: BGE, 10 mM sodium tetraborate; pH 9.0; hydrodynamic injection, 50 mbar for 12 s; applied voltage, 20 kV; detection wavelength, 195 nm.

3.2.1. Stability of solutions

BGE and drug solutions stability was evaluated under the refrigerated (4 °C) storage condition for a period of one month. The concentrations of freshly made solutions and those tested after a month period did not vary much (<1%).

3.2.2. Specificity

The specificity of the method was tested by adding a known quantity of standard drug solutions to the tablet excipients placebo solutions. The tablet excipients placebo solution (without drug) was prepared by mixing the common tablet excipients such as magnesium stearate, starch, lactose, and microcrystalline cellulose in deionized water with proportions of drug to excipients ratio. From the electropherogram of excipients alone

Table 3
Results of analysis of CTZ and CTZ composite pharmaceutical tablet

Commercial tablet	Actual ingredients	Labelled claim (mg)	Amount found (mg)	Recovery (%)	R.S.D. (%) ($n=5$)
Brand 1	CTZ	10	9.98	99.80	0.62
Brand 2	CTZ	10	10.12	101.20	0.86
Brand 3	CTZ	10	9.96	99.60	0.74
Brand 1	PPA	25	24.94	99.76	1.34
	PARA	500	499.18	98.84	0.56
	CTZ	5	4.98	100.10	0.42
Brand 2	PPA	25	24.88	99.52	1.56
	PARA	500	498.52	100.70	0.38
	CTZ	5	4.96	99.70	0.46
Brand 3	PPA	25	24.90	98.60	1.42
	PARA	500	498.22	99.64	0.36
	CTZ	5	4.97	100.30	0.50

(Fig. 3A) and the excipients spiked with the drugs (Fig. 3B) it is clear that no significant interference was found in the migration time of drug peaks during analysis.

3.2.3. Linearity

Linearity was assessed for three drug substances simultaneously in the combination standard mixtures with the concentration range of 2–50 μg ml^{-1} for CTZ, 10–1000 μg ml^{-1} for PARA, and 10–100 μg ml^{-1} for PPA. In all the above cases, 25 μg ml^{-1} of ibuprofen was added as I.S. Calibration curves were plotted from the standard drug concentrations versus peak area ratio (corrected peak area/I.S.) of individual drugs. The calibration curves were defined by the following equations: $y = 0.0072x - 0.0089$, $0.0069x + 0.1006$, and $0.0033x - 0.0074$; for CTZ, PARA, and PPA, respectively, where y is the peak area ratio with I.S. of individual drugs and "x" axis is the standard drugs concentration expressed in μg ml^{-1}.

3.2.4. Precision

Repeatability (intra-day) was tested with each five injections of three samples solutions containing lower, middle, and higher linearity range with a constant amount of I.S. (25 μg ml^{-1}). The results in Table 1 indicated that R.S.D. of migration time ≤0.22, ≤0.26, and ≤0.26% for (CTZ, PARA, and PPA) and peak area ratios ≤0.56, ≤0.42, and ≤1.46% for CTZ, PARA, and PPA, respectively.

Intermediate precision (inter-day) of the method was evaluated by considering lower, middle, and higher linearity range on seven consecutive days. The R.S.D. values of intermediate precision (≤4.92%) showed that the precision of the method was satisfactory (Table 1).

3.2.5. Accuracy

The accuracy of the proposed method was studied by recovery experiments. This was performed by adding 50, 100, and 150% of the nominal concentration of drugs standard solutions of CTZ, PARA, and PPA to the standard drug and pharmaceutical tablet solutions within the linearity range. Five samples were prepared for each recovery concentration level. The results obtained show excellent recovery of ≥98% for all the three drugs (Table 2).

3.2.6. LOQ and LOD

The LOQ defined as the lowest concentration that can be measured with acceptable precision and accuracy (S/N = 10) were 2 μg ml^{-1} (≤0.42% R.S.D), 2 μg ml^{-1} (≤0.38% R.S.D), and 4 μg ml^{-1} (≤1.68%R.S.D) for CTZ, PARA, and PPA, respectively. The LOD defined as the concentration where the signal to noise ratio of 3:1 were found to be 0.6, 0.6, and 1.0 μg ml^{-1} for CTZ, PARA, and PPA, respectively. The signal to noise ratio was calculated by using DAx software provided by the instrument manufacturer.

3.2.7. Robustness of the method

Robustness of the proposed method was tested by small but deliberate variations of pH (8.8–9.2), BGE concentration (8–12 mM) and applied voltage (18–22 kV). The deviations for corrected peak area and peak-to-peak resolution between these drugs (CTZ, PARA, and PPA) were ±2 and ±4%, respectively, from the optimized condition values.

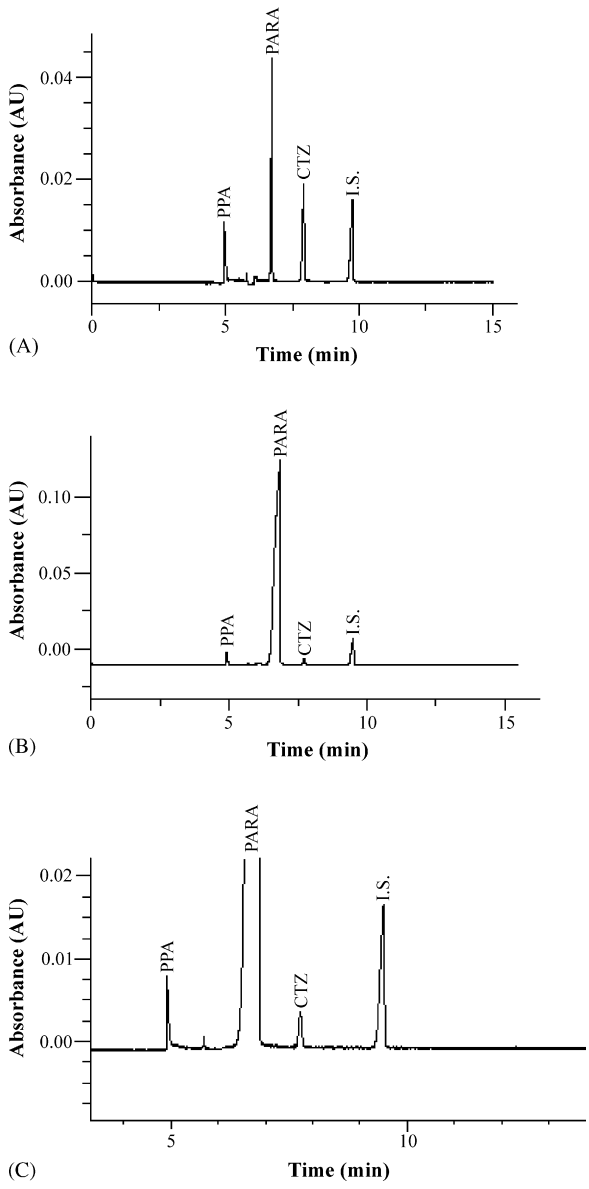

Fig. 4. Electropherogram of (A) CTZ, PARA, and PPA drug standard mixture of each 50 μg ml^{-1} with ibuprofen (I.S.) of 25 μg ml^{-1}, (B) Okacet tablet formulation containing CTZ, PARA, and PPA with I.S. (25 μg ml^{-1}) original electropherogram, and (C) Zoom in electropherogram. Electrophoretic conditions as given in Fig. 2.

3.3. Analysis of pharmaceutical tablets

3.3.1. Application to the determination of CTZ in tablets and simultaneous determination of CTZ, PARA, and PPA in composite tablets

The proposed method was applied for the determination of CTZ in tablet of different brands, and results were shown in Table 3. These data showed a recovery between 99.60 and 101.20% of label claim of tablet with acceptable precision (R.S.D. ≤ 0.86%, $n = 5$). The analysis results showed that the

tablet excipients did not interfere (figure not shown) with the analysis of CTZ in tablets.

The developed method was applied for the simultaneous determination of CTZ, PARA, and PPA in composite tablet (5 mg CTZ, 500 mg PARA, and 25 mg PPA per tablet) under the optimum conditions and results are shown in Table 3. The electropherograms of standard mixtures of each 50 $\mu g\,ml^{-1}$ and tablet solution of the three drugs CTZ, PARA, and PPA in the ratio of 1:100:5 with 25 $\mu g\,ml^{-1}$ of I.S. are shown in Fig. 4(A and B), respectively. From Table 3 it has been concluded that determination of lower concentration of drug in presence of high content is achieved with good recovery ($\geq$98%) and precision.

4. Conclusion

Under the optimized condition, baseline separation of all three drugs was achieved within 10 min with resolution >3.0. As compared with the reported HPLC method [3], the developed CZE method is less expensive, simple, rapid, eco-friendly (did not require any organic solvent in the mobile phase). The proposed CZE method is an alternative to HPLC method for routine analysis of PARA, CTZ, and PPA in pharmaceutical preparations.

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