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CE-C^{4}D method development and validation for the assay of ciprofloxacin

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ABSTRACT

A capillary electrophoresis method with capacitively coupled contactless conductivity detection (CE-C^{4}D) has been developed, optimized and validated for the determination of ciprofloxacin.

Ciprofloxacin is a member of the fluoroquinolone antibiotics with a broad spectrum bactericidal activity and recommended for complicated respiratory infections, sexually transmitted diseases, tuberculosis, bacterial diarrhea etc.

Method development was conducted with major focus on the quality by design (Q_bD) approach. During method development, multiple buffers were tried at different ionic strength. However, the optimized method finally involved a very simple background electrolyte, monosodium citrate at a concentration of 10 mM without pH adjustment.

The optimized CE-C^{4}D method involved an uncoated fused silica capillary (59/39 cm, 50 μm i.d.) and hydrodynamic sample injection at a pressure of 0.5 p.s.i. for 5 s. The actual separation was conducted for 10 min at normal polarity with a voltage of 20 kV corresponding to 5.9 μA current. LiCl (1 mg/mL) was used as an internal standard.

The optimized method is robust and accurate (recovery >98%) which rendered the ciprofloxacin peak within five minutes with good linearity ($R^2 > 0.999$) in the concentration range of 0.0126–0.8 mg/mL. The repeatability is expressed by percentage relative standard deviation (%RSD) of the relative peak areas (RPA) and it showed good repeatability both intra-day (<3%) and inter-day (3.1%). This method, proven to be free of matrix interference, showed that the estimated percent content of ciprofloxacin (102%) was within the official requirements.

Moreover, due to its ease of use and robustness, the method should also be applicable in less well controlled laboratory environments.

1. Introduction

Capillary electrophoresis (CE) is now-a-days a popular separation technique for ionic organic compounds including pharmaceuticals, biomolecules etc. The adoption of CE in the quality control of drugs in pharmaceutical industries is the prime focus for research groups working in this field around the globe. To date, CE enjoys widespread applications in fields like proteomics [1], genomics, metabolomics [2] due to the efficiency, resolution and straightforwardness it offers. Despite CE's potential as an analytical tool for drug quality control, liquid chromatography is still the preferred technique for routine pharmaceutical analysis. However, a different scenario is emerging with the hope of integrating CE in pharmaceutical quality control. The detection mode, ubiquitously integrated with most of the CE equipment is UV in addition to multiple choices like fluorescence, mass spectrometry etc. Another detection technique named capacitively coupled contactless conductivity detection (C^{4}D) is being used currently for the analysis of ionisable drugs [3]. This device works on the principle of measuring conductance difference between the background electrolyte (BGE) and the sample plug moving through the detector. It offers multiple benefits over UV detection. It is simple and has the flexibil-

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ity to change the effective length during the separation to improve resolution. Additionally, it can be used with capillaries made up of materials other than fused silica which made C^4D more flexible and has increased the scope of its usability [3]. It precludes the necessity of a fixed on-capillary detection window, thus ensuring prolonged capillary integrity. Moreover, the C^4D is able to detect both UV active and inactive ionized compounds. Besides, it is virtually maintenance free and requires no consumables.

The CE analytical tool has been proven to be a good alternative to liquid chromatography to determine drugs in pharmaceutical formulations. The addition of C^4D in the CE systems establishes a device which is affordable both technologically and economically. Therefore, optimism around deployment of this technique for quality control and prevention of counterfeit drugs in poor nations is increasing day by day. The combination of CE-C^4D can be considered as the economic and preferred technique for those nations not able to afford equipment with high maintenance and consumable cost.

CE-C^4D can be applied to almost every candidate drug enlisted in the World Health Organization (WHO) essential drug list and formulary [4] that is ionizable.

Among the essential drugs, antibiotics are considered critical due to the fact that poor quality and irrational use will result in a threatening medical phenomenon, called antibiotic resistance. The use of antibiotics among developing and underdeveloped countries is widespread due to prevailing infectious diseases and the deficiency in proper hygienic conditions as well as health awareness among the people. As a result, antibiotics are the most commonly prescribed medicine in those nations. The fluoroquinolone derivatives, for example, ciprofloxacin and its congeners are prescribed frequently in the developing world.

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinolonine-3-carboxylic acid) is a second-generation fluoroquinolone. Its spectrum of activity includes a widespread series of pathogens both Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli*, *Haemophilus influenzae*) [5]. It is commonly recommended for the treatment of urinary tract infections, upper respiratory tract infections and abdominal infections. It is also used as a prophylaxis for neutropenic patients [6]. The antimicrobial property of ciprofloxacin is attributed to inhibition of two nuclear enzymes, DNA gyrase and topoisomerase IV [7].

Quality control of any pharmaceuticals including ciprofloxacin involves determination of different parameters using instrumental techniques for both raw and finished pharmaceuticals [7]. Multiple works have already been performed for the analysis of fluoroquinolones by high performance liquid chromatography (HPLC) [8], spectrophotometry [9] and titrimetry [10].

CE has also been applied for the analysis of ciprofloxacin. Different detection modes have been coupled to CE, such as UV [11,12] and fluorescence [13]. Fluoroquinolones, especially ciprofloxacin, are UV active antibiotics (see chemical structure in Fig. 1). Hence, multiple CE-UV based methods [12,14] have been developed so far with diverse background electrolytes with or without organic solvents [7].

Application of the CE-C^4D system would be very interesting in the analytical method development for ciprofloxacin. The antibiotic ciprofloxacin is one of many drug candidates that has not been analysed by a CE-C^4D system so far. This study aims to develop and validate a CE-C^4D method for the determination of ciprofloxacin in raw as well as solid pharmaceutical formulations, such as tablets.

Besides, strong emphasis will be given to evaluate the effect of different instrumental (capillary temperature, voltage etc.) and methodical factors (BGE ionic strength, pH). The entire project was designed with twin aims in mind, namely, development of a very simple CE-C^4D method for ciprofloxacin in one hand and digging into the response variability in light of multiple, relevant factors associated with the system on the other hand. This practice will enable us to interpret experimental responses from the perspective of different factors studied. Consequently, we will have a better understanding about the method. That is, the method's quality will be built-in by the design of the experimentation (Q_bD).

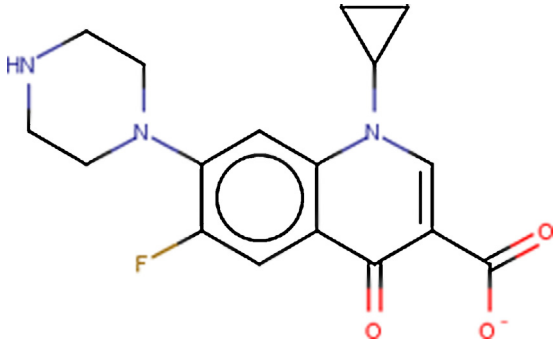

Fig. 1. Ciprofloxacin structure (pK_{a1} = 5.86, pK_{a2} = 8.24).

2. Material and methods

2.1. Reagents, samples and solutions

All chemicals used in this study were of analytical grade. Acetic acid and sodium hydroxide were from VWR Chemicals (Leuven, Belgium). Methanol was obtained from Acros Organics (Geel, Belgium). Reference standards of ciprofloxacin base were obtained from Acros Organics. The internal standard, lithium chloride (LiCl), was purchased from Acros Organics (Morris Plains, NJ, USA).

Several types of buffers were prepared during method development. Ammonium acetate was bought from Merck (Darmstadt, Germany). Anhydrous sodium dihydrogen citrate was obtained from Fluka Chemie (Buchs, Switzerland). MES and L-Histidine were bought from Sigma Aldrich (Steinheim, Germany). Sodium tetraborate decahydrate (Borax) was also obtained from Sigma Aldrich. Trishydroxymethylaminomethane (Tris) and L-arginine were obtained from Applichem (Darmstadt, Germany). Malic acid was sourced from Janssen Chimica (Beerse, Belgium).

The buffer solution was prepared by using Milli-Q water (Millipore, Milford, MA, USA) and filtered using syringes obtained from Filter service S.A. (Eupen, Belgium) and 0.45-μm membrane Chromafil Xtra PTFE-45/25 filters from Macherey-Nagel (Düren, Germany).

Standard solution of analyte was prepared by dissolving an accurately weighed amount of ciprofloxacin base directly into the diluted (10 x) BGE. Moreover, an aliquot of 0.5 M solution of HCl (Fisher scientific, Loughborough, UK) was added to dissolve the analyte properly. The sample solution was prepared from pulverized tablets (Neofloxin® 500, Beximco Pharmaceuticals Ltd., Bangladesh) in the same way as standard solution. A similar filtration approach as for the BGE, was applied for the sample and standard solution before injection.

2.2. Instrumentation and operating conditions

CE experiments were performed on a P/ACE MDQ (Beckman Coulter, Inc. Fullerton, CA, USA). An eDAQ C^4D device (eDAQ, Denistone East, Australia) was connected to the CE instrument as a signal recorder. Data recording and processing was done by PowerChrom v2 (eDAQ) and 32 Karat™ 4.0 (Beckman Coulter). PowerChrom v2 was mainly used for data recording, processing and detector

module control. 32 Karat™ 4.0 software was used as a controlling unit.

The capillaries were uncoated fused silica capillaries obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary dimension was 59 cm long, 39 cm to the detector with 50 μm of internal diameter (i.d.).

The settings of the C^4D are dependent on the specific BGE used. For each BGE used, a C^4D-profiling was done to find the necessary parameters. In case of the ammonium acetate buffer, the eDAQ C^4D detector was employed at a peak-to-peak amplitude of 60 V and an input frequency of 1050 kHz. For the citrate buffer the eDAQ C^4D detector was employed at a peak-to-peak amplitude of 40 V and an input frequency of 800 kHz.

Separation voltage, capillary temperatures, injection mode and time as well as capillary rinsing condition were optimised by the trial and error approach and also determined based on previous research. New capillaries were conditioned at 25 °C by rinsing with 1 M NaOH (10 min), Milli-Q water (10 min), 0.1 M NaOH (10 min) and again Milli-Q water (10 min). Daily at the start of analysis, the capillary was rinsed with 0.1 M NaOH, Milli-Q water and BGE for 10 min each. In between runs the capillary was rinsed with BGE for 3 min.

Every evening, the capillary was rinsed with water for 10 min to clean it. Weekly, the two platinum electrodes were cleaned thoroughly with a methanol-water mixture (50:50, v/v) using a cotton swab.

2.3. Experimental design

For the robustness evaluation of the optimized method, a two level fractional factorial design was applied including the central value. The total number of experimental runs including replicates was $2^{k-1} + n$, where 'k' is the number of factors and 'n' is the number of central points. All the experimental runs were done in random order. The quantitative relationship between individual and interacting factors is given by the following equation:

$$Y = \beta_o + \beta_i X_i + \beta_j X_j + \ldots + \varepsilon$$

Where β_o, β_i, β_j, β_{ij}...are the regression coefficients and ε is the residual of the experimental data. All the coefficients basically describe the importance of individual (β_i, β_j) factors on the response variable RPA.

The statistical significance of the variables was analysed by the open source R statistical software package and carried out at a 95% confidence limit.

3. Results and discussion

3.1. Method development

Initially, finding a buffer suitable for analysis of ciprofloxacin constituted the primary study of this work. Selection of the buffer was based on the careful study of the physicochemical properties of the analyte (ciprofloxacin) and relevant literature search. Ciprofloxacin has two pK_a values of 5.86 and 8.24 [14]. According to the European Pharmacopoeia, the ciprofloxacin base (see Fig. 1) is insoluble in water. It is, however, soluble in dilute hydrochloric acid.

A general knowledge of the pK_a of the analyte and the pH of the BGE was applied initially for the selection of the BGE. Theoretically, ciprofloxacin will be cationic and anionic in a BGE with low and high pH, respectively. In the early stage of the study, Peakmaster 5.1 was used as a tool to improve the efficiency of identifying a suitable BGE for the analysis of ciprofloxacin [15]. The BGE scouting was based on buffer criteria suitable for maximum C^4D sensitivity and ease of preparation, for example, compounds with low conductivity (organic acids and bases) that are easily available and stable at ambient climate. Table 1 lists the number of BGEs scouted.

The Citric acid-L-arginine (pH 4.5; 20 mM each) and Malic acid-L-arginine (pH 4.5; 8 and 5 mM respectively) buffers were used at the start of the research. However, failure to find the ciprofloxacin peak coupled with a high baseline noise prompted to search for other BGEs.

The MES-Histidine (pH 7.51; 20 mM each), Borax (pH 9.27; 5 mM) and Tris (pH 10.4; 4 mM) buffers were tried in reversed polarity mode. Since basic media convert ciprofloxacin into an anion, reversed polarity of the electric field was necessary to detect the analyte in the existing instrumental set-up. Unfortunately, no ciprofloxacin peak appeared.

A further study involving ammonium acetate as buffer identified the ciprofloxacin peak in both the UV and C^4D. Two different concentrations (10 and 20 mM) were investigated for good baseline stability and better analyte sensitivity. However, the peak appearing with either concentration of ammonium acetate buffer was accompanied by high baseline noise leading to a high variability in RPA of ciprofloxacin.

Finally, a study involving a simple buffer, monosodium citrate at 10 mM concentration and a pH of 3.85 exhibited a good C^4D response with satisfactory baseline stability. Good sensitivity, peak shape and separation from possible interfering peaks were obtained with this citrate buffer in comparison to the ammonium acetate buffers. There was no Joule heating over the applied voltage range of 10–30 kV (Ohm's plot determination coefficient = 0.9991). An example of a CE-C^4D electropherogram for ciprofloxacin using citrate buffer at 20 kV is visualized in Fig. 2.

In practice, the ciprofloxacin was dissolved at a concentration of 1 mg/mL in diluted BGE (10 x) with few drops (400 μL) of hydrochloric acid (0.5 M).

After BGE selection, an appropriate capillary rinsing protocol was established to achieve good repeatability. Between run rinsing with buffer (2, 3, 5 min), 0.1 M NaOH (5 min) followed by water (5 min) and BGE (4 min) were evaluated against repeatability. However, washing the capillary with BGE for 3 min was found the most effective. Moreover, incorporation of lithium chloride (LiCl) as internal standard (IS) was also studied to further improve the repeatability of this method.

CE-C^4D experimentation was also carried out at different voltages (10–30 kV) and corresponding currents with and without capillary temperature control. The migration of analyte was observed faster at higher voltage but the migration time ratio was found almost similar indicating no significant loss in resolution between internal standard and analyte.

Moreover, the impact of temperature on the CE-C^4D analysis of ciprofloxacin was evaluated by conducting the experiment at different temperatures. The results indicate that increasing the temperature is associated with a decrease in MT while keeping the resolution similar. The percentage relative standard deviation

Table 1
Buffers screened during method development (NS = no significant result, MT = migration time).

Buffer	pH	UV MT (min)	C^4D MT (min)
20 mM Citric acid + 20 mM L-Arginine	4.50	6.3	NS
20 mM Ammonium acetate	4.50	4.4	3.3
10 mM Ammonium acetate	4.50	4.3	3.2
20 mM MES + 20 mM Malic acid	4.50	5.7	NS
13 mM Malic acid + 20 mM L-Arginine	4.50	6.6	NS
8 mM Malic acid + 5 mM L-Arginine	4.50	4.9	NS
10 mM Monosodium citrate	3.85	2.2	3.0
5 mM Borax	9.27	5.7	NS
4 mM Tris	10.4	3.4	NS

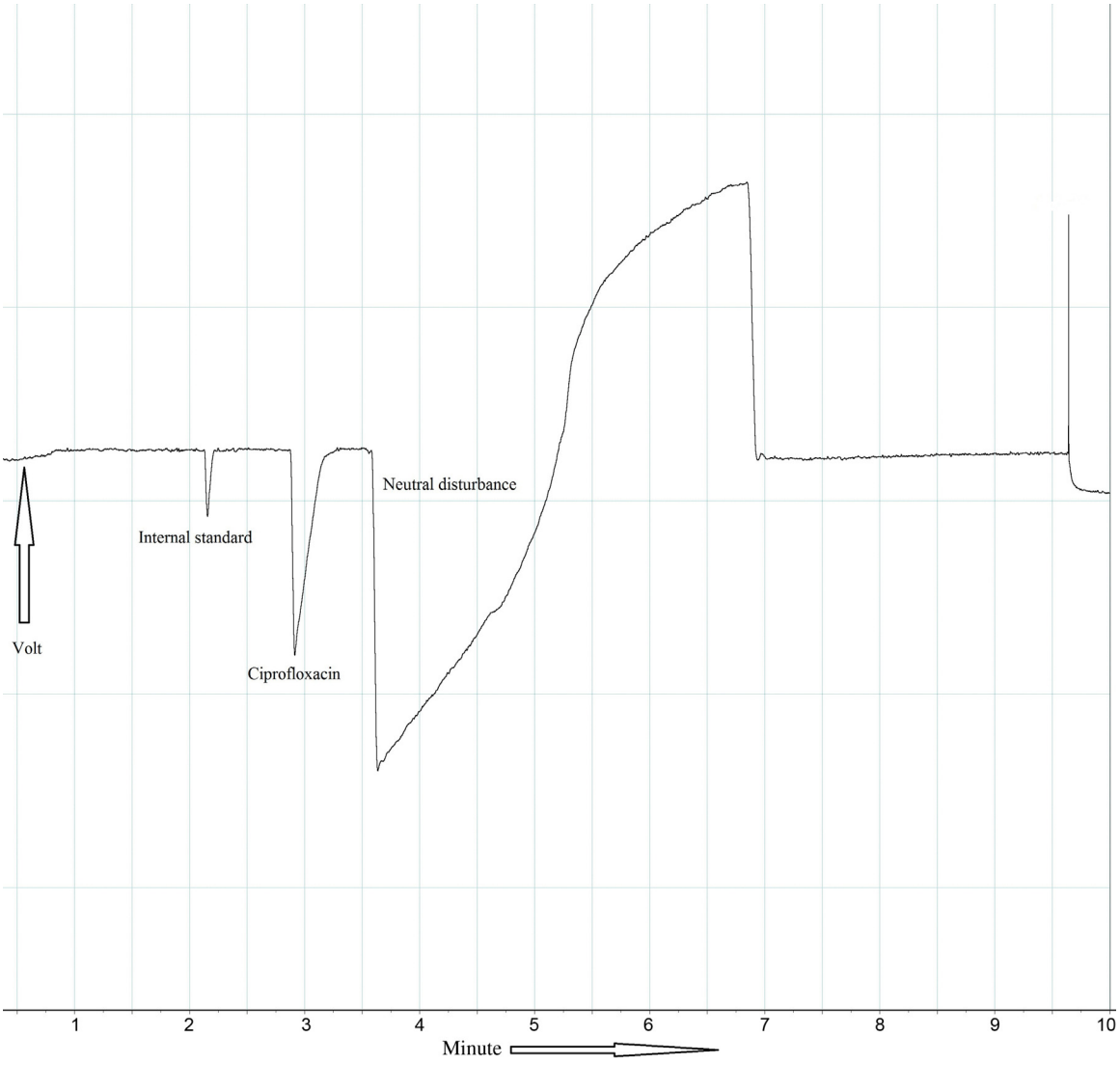

Fig. 2. Electropherogram of Ciprofloxacin (0.5 mg/mL) and Li^+ (internal standard).

Table 2
Temperature impact on MT and RPA repeatability.

Temperature (°C)	Mean MT (min)	Mean RPA	%RSD
15	5.6	2.356	3.1
20	5.1	2.486	3.7
25	4.5	2.453	2.9
30	4.2	2.355	3.1

(%RSD) values of RPA were close to 3% with precise mean RPAs at all temperatures as shown in Table 2.

3.2. Constant current electrophoresis

Reports from previous studies concluded that better linearity and repeatability profiles were obtained by shifting from constant voltage (20 kV) to the corresponding constant current (5.9 μA) [16]. A comparison of the results in terms of%RSD of MT and RPA for both separation modes was performed to observe the benefits from one versus the other. As stated by Altria and Fabre [17], the precision of a CE method can be improved by applying a constant current instead of using a constant voltage. The main advantage of using constant current over constant voltage is decreased temperature fluctuation. The results from this experiment showed a statistically insignificant difference between both CE modes. The%RSD of RPA was 2.3% at constant voltage (20 kV) and 2.5% at constant current (5.9 μA). This may be attributed to the efficient temperature control by the liquid coolant, the low buffer concentration and the moderate separation voltage applied. Therefore, CE was conducted at constant voltage for the further method optimization and validation.

3.3. Impact of water quality on RPA

The buffer composition is crucial in the experimental set up of CE-C^4D analysis. Consequently, the water quality being used for preparation of the BGE might have an influence on the response (RPA) of ciprofloxacin. To answer this query, an experiment was performed using the same BGE (10 mM monosodium citrate) prepared with ultrapure grade water (Milli-Q) and demineralized (DM) water while keeping the rest of the CE-C^4D conditions constant. Six replicates of standard ciprofloxacin solution (0.5 mg/mL) with 200 μL IS (1 mg/mL) were carried out per BGE and RPAs were calculated. The averages of the RPAs were compared through Student's *t*-test for statistical significance at 95% confidence limit. A 'p' value of less than 0.05 was considered statistically significant.

The independent sample *t*-test revealed no significant impact ($p > 0.05$) of the water quality on the mean RPA of ciprofloxacin

peak. Hence, this experiment can be done in a laboratory lacking ultra-pure grade water, for example, those from developing and under-developed nations.

3.4. Method validation

To properly validate the method for CE-C^4D analysis of ciprofloxacin, method validation was performed according to the International Conference on Harmonisation (ICH) guidelines [18,19]. The study was carried out in terms of limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy and linearity. Moreover, an internal standard was used in every validation related study to address the repeatability issue due to injection variability.

3.4.1. Selectivity

A comparative study between the slope of a calibration plot and that from a standard addition plot was carried out to assess the selectivity of the method, because of the lack of placebo for the active principle [19]. Both calibration and standard addition plot were constructed at five concentration levels of ciprofloxacin (0.2, 0.3, 0.4, 0.5 and 0.7 mg/mL) with three replicates each and compared statistically [7]. The results of Table 3 show the statistical (*t*-test) similarity ($p > 0.05$) of the slopes.

3.4.2. Sensitivity

The sensitivity of the method is expressed in LOD and LOQ. The LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. ICH defines the LOQ as the lowest amount of analyte in a sample which can be detected quantitatively with adequate precision and accuracy [18].

The detection limit was calculated based on the graphically determined signal-to-noise ratio (S/N) of the ciprofloxacin peak. The concentration corresponding to a S/N of 3 was considered as LOD while that of 10 was taken as LOQ. In this study, the LOD and LOQ values for ciprofloxacin have been calculated for two BGEs. The results demonstrated reasonably low LOD and LOQ in citrate BGE (see Table 4).

3.4.3. Robustness

The International Conference on Harmonization (ICH) defines the robustness of an analytical method as its ability to withstand small but deliberate changes in the experimental variables. In this study, the response is measured as a function of variables involved and the changes made deliberately during robustness testing mirror the changes that may occur while performing the same experiment at different environments, different laboratories or by different personnel etc. [20,21].

Robustness of a method is evaluated preferably by an experimental design to examine the influence of different variables simultaneously on the response(s) [22].

Table 3
Statistical results from selectivity study.

Plot name	Slope	R^2	t-test	p-value
Calibration	11.794	0.997	−3.05	>0.05
Standard addition	11.799	0.999		

Table 4
LOD and LOQ values of ciprofloxacin in two BGEs.

Buffer	LOD (mg/mL)	LOQ (mg/mL)
10 mM Sodium citrate	0.00425	0.0125
20 mM Ammonium acetate	~0.450	~1

Table 5
CE factors at different levels with corresponding values.

CE parameter	Low level (−)	Central level (0)	High level (+)
BGE pH	3.8	3.85	3.9
Temperature (°C)	23	25	27
BGE concentration (mM)	9.5	10	10.5

In this study, a two level fractional factorial design was adopted as part of the robustness evaluation. The aim of this technique was to identify critical factor(s) on the responses. The RPA and migration time ratio (MTR) of ciprofloxacin were considered the responses in this study, since the purpose of the method was quantitative analysis of ciprofloxacin only. Three factors, namely, pH of the BGE, concentration of the BGE and temperature were varied at two levels: low (−), and high (+) levels. Three central points were also included, thus making the total number of runs of $2^{3-1} + 3 = 7$, with three replicates at each experimental condition (see Table 5). The pH of the BGE was adjusted to low and high value by citric acid (5 mM) and sodium hydroxide (0.1 M) respectively.

Randomization of the experimentation was performed to avoid the effect of time order of the experimental runs. The data were analysed statistically by the application of analysis of variance (ANOVA) using the R statistical software package. To differentiate among factors in terms of RPA, statistical analysis revealed no significant impact of either of the factors ($p > 0.05$). Likewise, the other response (MTR) has also exhibited good robustness ($p > 0.05$) at the experimental parameters evaluated.

The effect of the variation of experimental factors on the responses (RPA and MTR) has also been evaluated by coefficient plots (see Figs. 3 and 4). The position of the points corresponding to each factor denotes the estimated effects by the distance of this point to the zero line. This distance can be positive or negative. The 95% confidence interval is depicted by the fine error line. When this error line crosses the zero line, the respective factor is considered not significant. The bold error line represents the 50% confidence interval. It has been observed that the 95% error lines of the effects resulting from the factors temperature and pH cross the zero line, indicating no significant impact on the calculated responses, RPA and MTR (see Figs. 3 and 4). This finding has been consolidated by the conclusion derived from the ANOVA analysis of experimental data ($p > 0.05$ for all factors). However, the coefficient plot for RPA suggests the necessity of accurate weighing of BGE components.

3.4.4. Linearity

It is very important to establish a linear relationship between the analyte concentration and the corresponding response. The linearity of the method was assessed by the coefficient of determination (R^2) of the calibration curve. Six concentration levels of standard solution were prepared by diluting the standard stock solution of ciprofloxacin (1 mg/mL) giving rise to concentrations of 0.0126, 0.0628, 0.129, 0.258, 0.516, 0.86 mg/mL of ciprofloxacin. Standard solutions from each concentration level were injected three times and analysed by CE-C^4D. The construction of a calibration plot of mean RPA at six concentration levels versus the corresponding concentration showed that the linear model fits well ($R^2 > 0.999$ for the three series, run on three days) to the data with good%RSD (0.2–2.3%) in the concentration range 0.0126–0.86 mg/mL.

Moreover, it was also noted that the 95% confidence interval of the intercept included zero (data not shown). In addition, a residual plot was produced to evaluate the appropriateness of linear regression to fit the data. Since the points were distributed randomly around the horizontal axis, it was concluded that linear regression is suitable for these data.

However, it was observed that a ciprofloxacin concentration exceeding 1 mg/mL tends to broaden at the base of the peak and the

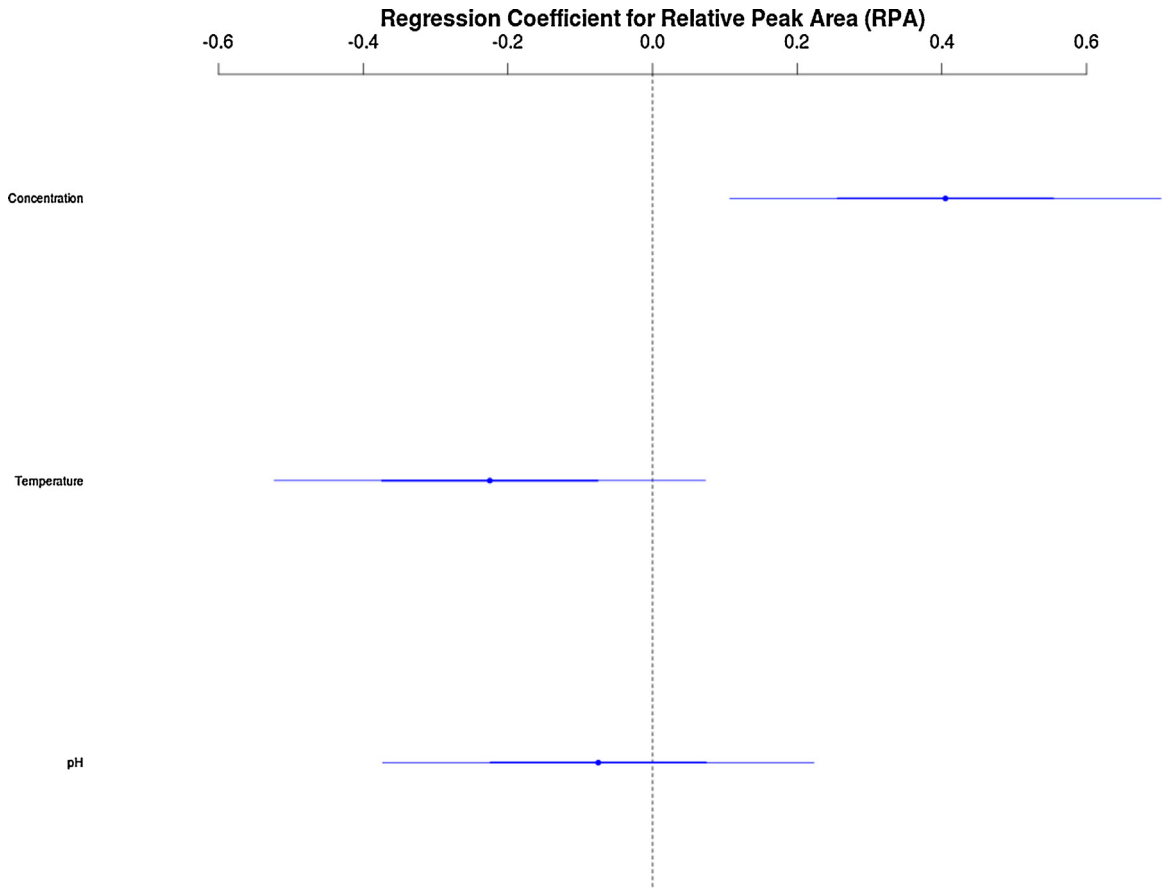

Fig. 3. Coefficient plot for relative peak area (RPA).

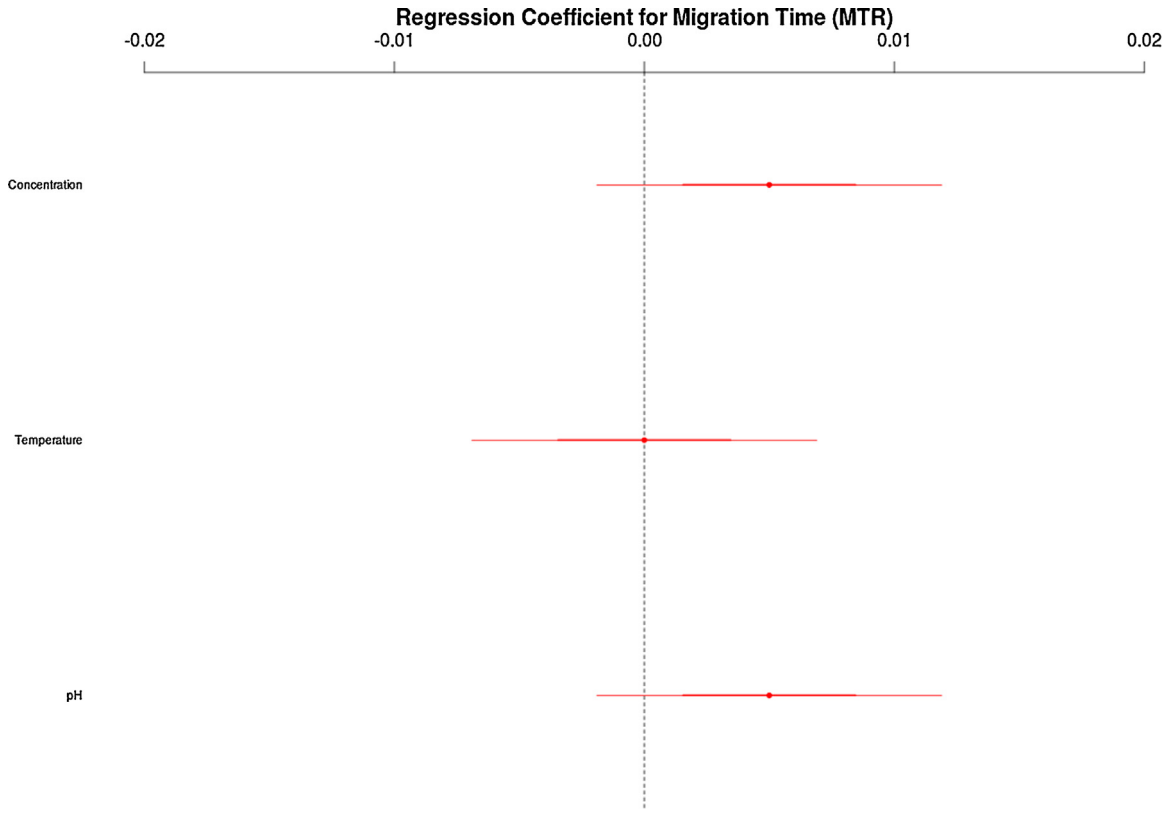

Fig. 4. Coefficient plot for migration time ratio (MTR).

resolution between ciprofloxacin and the EOF disturbance diminishes.

3.4.5. Accuracy

The accuracy of a method measures the degree of closeness of the estimated value to the true value. In this experiment, the accuracy was determined based on the recovery of analyte at the LOQ. Due to the lack of placebo for the commercial sample, a sample solution was considered as sample background. Here, a certain volume of sample solution corresponding to the LOQ was spiked at three levels (80%, 100% and 120%) with a standard solution. In parallel, equivalent levels of standard solution were prepared and analysed. The IS was introduced in all the solutions.

The accuracy of the method was calculated by applying the following equation:

$$\%\text{Recovery} = \frac{RPA_{a+s} - RPA_a}{RPA_s}$$

where RPA_{a+s} refers to RPA from standard addition and RPA_a corresponds to sample RPA while RPA_s is designated as RPA of standard solution. The experimental results showed the recovery within the range of 98.8% to 103.1% for the sample analysed. This observed variability in recovery could be due to inherent uncertainty associated with CE analysis. Additionally, variability in the sample preparation might have contributed to the variation of recovery of this method.

3.4.6. Precision

The precision of the method was evaluated both intra-day and inter-day. It was expressed as the%RSD of RPA and MT from six replicate injections of 0.5 mg/mL standard solution. Lower concentration levels including the LOQ, were also injected on three consecutive days. The experimental data showed that the intra-day and inter-day%RSD values were always below 3% and 2% for RPA and MT, respectively (data not shown). Only the intermediate precision at the LOQ level yielded a RSD value of 3.1% for RPA. Moreover, different concentrations of ciprofloxacin showed good precision for RPA in the calibration curve (see section 3.4.4).

Repeatability was also tested with the application of constant current (CC) and constant voltage (CV). The standard concentration (0.5 mg/mL) was prepared and analysed six times with CC or CV in the same day. CC may reduce variable heat generation inside the capillary thereby acting against radial and axial temperature fluctuation which can lead to more precise results. The difference in%RSD of RPA between the two modes is relatively low (see section 3.2.) and it is difficult to draw any conclusion if running on CC is more precise or not. Both applications are repeatable at the standard concentration of 0.5 mg/mL.

3.5. Quantification by calibration

As final part of this study, the optimized and validated CE-C^4D method was applied to determine the percent (%) content of ciprofloxacin in a commercial sample (500 mg/tablet).

For the assay, three tablets were taken randomly, weighed on an electronic balance and ground to fine powder by mortar and pestle. Afterwards, a solution of the sample was prepared by dissolving a certain amount of sample powder into 10 times diluted BGE followed by sonication for five minutes. The RPA values from the experiments were fitted into the calibration plot from the linearity study. The assay value for that batch of the tablets was found to be 102% of the label claim.

Additionally, as the nature of the composition of the sample matrix was unknown, a standard addition technique was also applied to compensate for any unknown interference of sample matrix on the experimental results. Both quantitative techniques showed that the calculated% content of ciprofloxacin in the sample (102%) is within the compendial acceptable limit of 95%-105%.

4. Conclusion

From the experimental data, it can be concluded that a CE-C^4D method for the analysis of ciprofloxacin has been developed, optimized and validated which is very simple and straightforward to perform, robust, relatively cheap and user friendly. An extensive study of the effect on the response(s) of different possible factors led to a better understanding of the method performance. In other words, the quality of this analytical method has been ingrained into the design of the experiment. Another good aspect of this CE-C^4D is the absence of rigid requirements of skilled personnel. This method was developed resulting in an experimental protocol which minimizes the complexity in routine application.

In comparison with the previously published CZE methods for determination of ciprofloxacin in pharmaceutical formulations [7,14], this method offers a short analysis time and a fairly simple BGE. Unlike HPLC, the method requires very small amounts of BGE and sample. Moreover, it is free of any organic solvent which makes it a green technique too. Additionally, the full automation with short analyses makes this method time efficient as well. This CE-C^4D method is user friendly and flexible compared to CE-UV in a sense that capillary assembly is easy and no optical window is needed. Hence, capillary integrity is maintained for a longer time.

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