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Determination of antazoline and tetrahydrozoline in ophthalmic solutions by capillary electrophoresis and stability-indicating HPLC methods☆

Mehmet Gumustas[a], Usama Alshana[b,c], Nusret Ertas[b], Nilgun Gunden Goger[b], Sibel A. Ozkan[a], Bengi Uslu[a,*]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey
[c] Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, 99138 Mersin 10, Turkey

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ABSTRACT

Capillary electrophoretic (CE) and high performance liquid chromatographic (HPLC) methods were developed and optimized for the determination of antazoline (ANT) and tetrahydrozoline (TET) in ophthalmic formulations. Optimum electrophoretic conditions were achieved using a background electrolyte of 20 mM phosphate buffer at pH 7.0, a capillary temperature of 25 °C, a separation voltage of 22 kV and a pressure injection of the sample at 50 mbar for 17 s. HPLC analysis was performed with Kinetex (150 × 4.6 mm ID × 5 μm) (Phenomenex, USA) analytical column with 1 mL min^{-1} flow rate of mobile phase which consisted of 0.05% TFA in bidistilled water (pH adjusted to 3.0 with 5 M NaOH) and acetonitrile/buffer in the ratio of 63:37 (v/v) at room temperature. Injection volume of the samples was 10 μL and the wavelength of the detector was set at 215 nm for monitoring both analytes.

Calibration graphs showed a good linearity with a coefficient of determination (R^2) of at least 0.998 for both methods. Intraday and interday precision (expressed as RSD%) were lower than 2.8% for CE and 0.92% for HPLC. The developed methods were demonstrated to be simple and rapid for the determination of ANT and TET in ophthalmic solutions providing recoveries in the range between 97.9 and 102.70% for CE and HPLC.

1. Introduction

Antazoline (ANT) (Scheme 1a) is a histamine H1 receptor antagonist. It can prevent histamine from acting on target cells through a reversible competition effect on histamine receptor sites of those cells [1–4]. It is classified as a first generation antihistamine having anticholinergic properties used to reduce nasal congestion and in eye drops, usually in combination with naphazoline and/or tetrahydrozoline (TET) (Scheme 1b) to reduce the symptoms of allergic conjunctivitis. TET is a derivative of imidazoline that is found in over-the-counter eye drops and nasal sprays. It is a sympathomimetic agent with α-adrenergic activity and its main mechanism of action is the constriction of conjunctional blood vessels and it acts as a local vasoconstrictor. This serves to reduce the redness of the eye caused by minor ocular irritants. Solutions and suspensions of TET are used as a conjunctival decongestant [1–5]. A combination of these two drugs is now available in the pharmaceutical market for ophthalmic use in the world such as Germany, India, Nigeria, Malaysia, etc.

High-performance liquid chromatography (HPLC) is commonly reported for the determination of ANT and TET [6,7]. Capillary electrophoresis (CE) is a complementary technique to HPLC which offers several unique characteristics that make it particularly attractive, which include its high resolving power, minimal reagent consumption, rapidness and low analyses cost.

To the best of our knowledge, neither CE nor stability-indicating HPLC method can be found in literature for the simultaneous determination of ANT and TET in pharmaceutical dosage forms. A few methods using HPLC [6,8] and only one high-performance thin layer chromatography method [9] have been reported for the simultaneous determination of these drugs. The proposed method describes the optimization of the two methods and it presents the effect of core-shell particles on the chromatographic separations.

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* Corresponding author at: Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Tandogan/Ankara, Turkey.
E-mail address: buslu@pharmacy.ankara.edu.tr (B. Uslu).

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Core–shell particles are the best-suited materials for rapid analyses because their optimum linear velocity is significantly higher than that of fully porous particles. On the other hand, these particle-packed columns show a pressure drop that can be half or even less than that of fully porous packed columns. This type of material provides higher plate number (efficiency) than fully porous stationary phases under the same conditions and with the same instrumentation. As a result of the higher efficiency, better resolution, narrower and more symmetrical sharp peaks can be observed. Analyzing complex matrices and impurity-profiling studies require that the method be sensitive [10,11]. In addition, consumption of organic solvents should be minimized for the method to be inexpensive and to fit into green chemistry regulations. Accordingly, core-shell materials can be ideal for quality control and research and development laboratories, where cost and time are of essential consideration, since they decrease the run times per analysis.

The goal of this study is to present the development and validation of rapid and easy CE and stability-indicating HPLC methods for the simultaneous determination of ANT and TET in raw materials and pharmaceutical preparations. The proposed methods offer automatic sample processing, as well as improved selectivity and efficiency that may be adequate for sensitive pharmaceutical studies with small sample size in addition to minimum consumption of organic solvents, symmetrical peaks and high resolution values. Both methods were validated according to the United States of Pharmacopeia (USP 32) and guidelines of the International Conference on Harmonization (ICH) [12–15]. Forced degradation studies are also presented to show the stability-indicating capacity of the developed HPLC method.

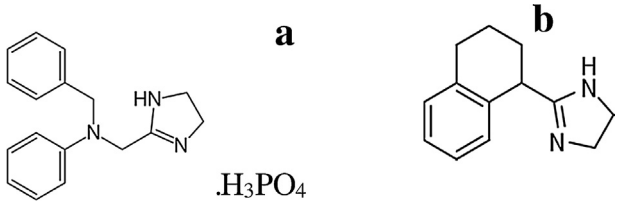
2. Materials and methods

2.1. Chemicals and reagents

ANT phosphate, TET Hydrochloride and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quetiapine that was used as an internal standard (IS) for HPLC study was kindly supplied from Mustafa Nevzat Ilac San. Tic. A.S. (Istanbul, Turkey). Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), trifluoro acetic acid (TFA) and hydrogen peroxide (H_2O_2) were purchased from Merck (Darmstadt, Germany). All reagents were at least of an analytical grade. Deionized (DI) water (18.2 MΩ.cm) which was treated with Millipore (Simplicity, 185 water purification system) was used unless otherwise stated.

2.2. Equipment and conditions

HP^{3D} CE (Agilent Technologies, Waldbronn, Germany) equipped with an online diode-array UV detector (DAD) was operated at a wavelength of 192 nm for monitoring the analytes, an optimum wavelength as determined using 'Isoabsorbance' and '3D' plots in the instrument's 'Data Analysis' software (Agilent Technologies, Waldbronn, Germany), was used. Separations were achieved using an uncoated fused-silica capillary (Agilent Technologies, USA) of 75 μm i.d. and 64.5 cm total length with effective length to the detector of 56 cm.



Scheme 1. Chemical structures of ANT (a) and TET (b).

Agilent Technologies 1100HPLC system (Wilmington, USA) was used for method development, forced degradation and method validation studies. This system equipped with a G1379A degasser, G1311A quaternary pump, G1313 auto injector and G1315B DAD. The chromatograms were recorded and the peaks were quantified and integrated using Chemstation® software and pH of the all buffer solutions was measured with a Thermo Scientific Benchtop pH meter (Orion 3 Star™ Plus, USA) using a combined electrode with an accuracy of ±0.05 pH.

HPLC analyses were performed with Kinetex (150 × 4.6 mm ID × 5 μm) (Phenomenex, USA) analytical column that have core-shell particles. A flow rate of 1.0 mL min^{-1} was applied for pumping of the mobile phase which consisted of 0.05% TFA in bidistilled water (pH adjusted to 3.0 with 5 M NaOH) and acetonitrile in the ratio of 63:37 (v/v) at room temperature. Samples were injected with the volume of 10 μL and the wavelength of the detector was set up at 215 nm.

2.3. Sample preparation

2.3.1. Standard and working solutions

Individual stock solutions of ANT and TET were prepared in methanol at a concentration of 2000 μg mL^{-1} and stored at −15 °C. Mixed standard solutions were freshly prepared from the stock solutions by proper dilutions with DI water for CE experiments. For HPLC study, stock solutions of ANT and TET were prepared as (1000 μg mL^{-1}) and IS was prepared in methanol at 100 μg mL^{-1} in ultrasonic bath for 10 min and was then kept in the darkness at 4 °C. Working solutions were prepared by dilution of the stock solutions in the mobile phase.

2.3.2. Preparation of forced degradation solutions

The stability-indicating were performed under alkaline, acidic, oxidative, photolytic and thermal stress conditions at a final concentration of 50 μg mL^{-1}.

Forced degradation was achieved by treating the stock solution with 0.1 M and 1 M HCl for acidic hydrolysis, 0.1 M and 1 M NaOH for alkaline hydrolysis, and 3% and 30% H_2O_2 for oxidative degradation for both analytes. The solid form of the analytes was exposed to UV light at 254 nm and room temperature, and thermal degradation was realized in an oven that was adjusted 100 °C for 6 and 24 h. Purity results of each peak were then screened on the software.

2.3.3. Analysis of pharmaceutical dosage forms and recovery assay

For the analysis of pharmaceutical preparations with HPLC, Allergoconjunct® solution which contained ANT at 0.15 mg mL^{-1} and TET at 0.50 mg mL^{-1} was diluted with methanol to the mark in a 25-mL volumetric flask. The contents of the flask were sonicated for 10 min to achieve complete dissolution. Appropriate solutions were prepared by taking suitable aliquots and diluting with the mobile phase. Concentration of the IS was kept constant in all dilutions and the chromatographic procedure was applied. For CE experiments, diluted solutions of the above-mentioned formulation were freshly prepared by proper dilutions with DI water.

2.4. Validation of the methods

HPLC and CE methods were validated to quantify ANT and TET in the commercial formulation according to parameters such as specificity, linearity, linear dynamic range (LDR), precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). Prior to the validation studies, the separation conditions were carefully optimized. To do so, system suitability tests were performed

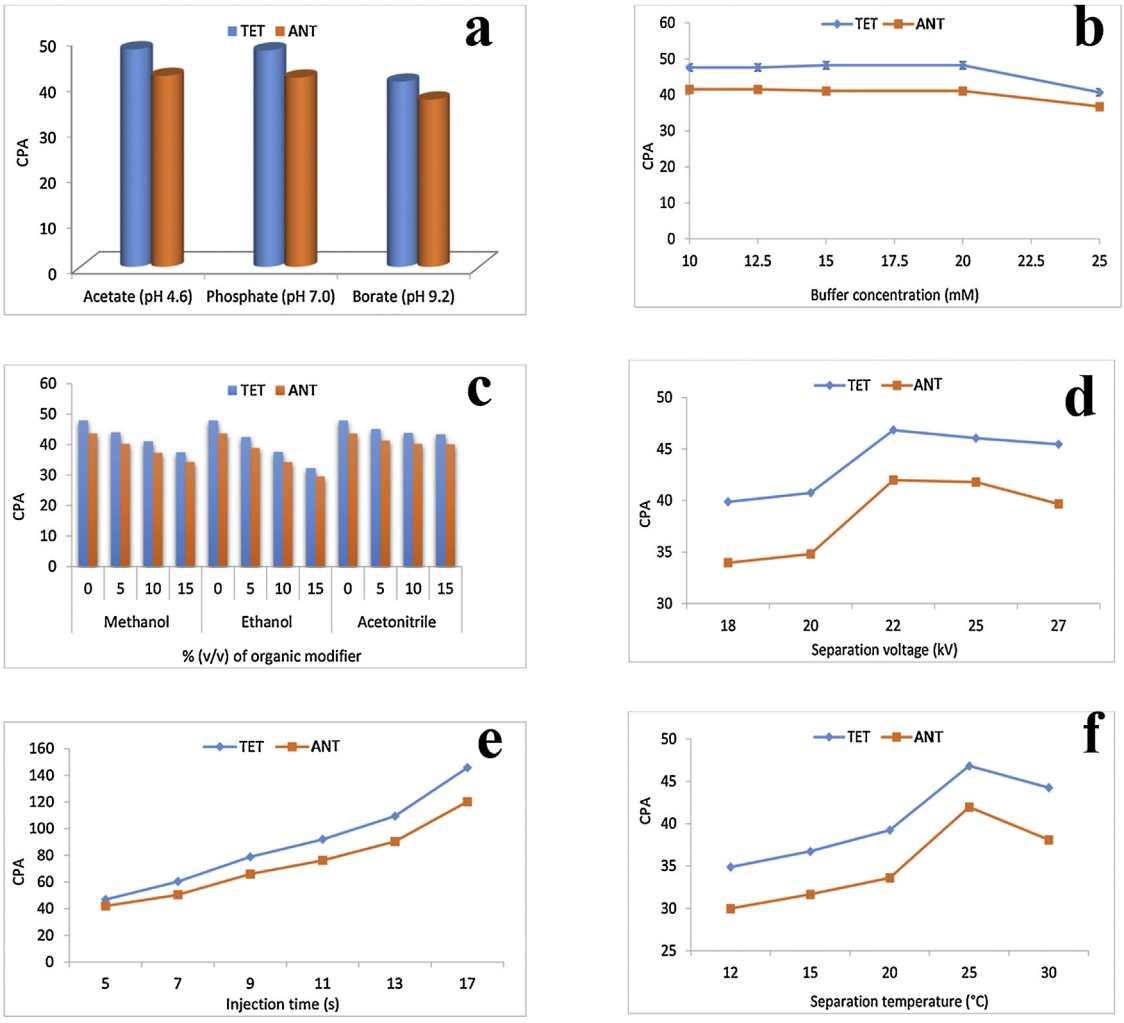


Fig. 1. Optimization of capillary electrophoresis parameters. Effect of buffer type (a), Effect of buffer concentration (b), Effect of organic modifier (c), Effect of separation voltage (d), Effect of separation temperature (e) and Effect of injection time (f).

Table 1
Parameters of system suitability tests.

Technique	HPLC			CE	
Compounds	TET	IS	ANT	TET	ANT
Retention/Migration Time	1.785	2.819	3.165	3.80	4.05
Selectivity Factor (α)	–	1.580	1.120	–	1.07
Resolution Factor (R_s)	–	7.330	2.190	–	6.370
Theoretical Plate Numbers (Plate/column)	3369	5053	6525	114770	231831
RSD% of Retention Time[a]	0.047	0.135	0.149	0.850	0.798

[a] Each value is the mean of five experiments.

in order to ensure that the method can generate results of acceptable accuracy and precision. Test parameters included theoretical plate number, resolution factor and selectivity.

2.4.1. Specificity

Specificity of the method can be described based on the ability of the method to verify the analyte in the presence of excipients, matrix and impurities [15–17]. According to the obtained chromatograms, the pharmaceutical preparation and its placebo (mixture of the active pharmaceutical ingredient (API) and excipients) showed almost no interfering peaks within the retention time ranges. In addition, forced degradation studies were performed to prove the specificity of the chromatographic method.

2.4.2. Linearity and linear dynamic range

In CE studies, concentrations of the analytes were varied within the range of 5.3–100 μg mL^{-1} and 2.3–125 μg mL^{-1} for ANT and TET, respectively. In HPLC, wider LDRs were achieved within the concentration range of 0.5–200 μg mL^{-1} for both analytes. Calibration graphs were constructed by plotting the ratio of the peak area of the drug to that of the IS against the active pharmaceutical ingredient (API) concentration for HPLC and plotting concentration versus peak area for CE with realizing repeated analysis (It was checked over the same concentration range on three consecutive days). The linear regression was demonstrated and tabulated by the necessary parameters.

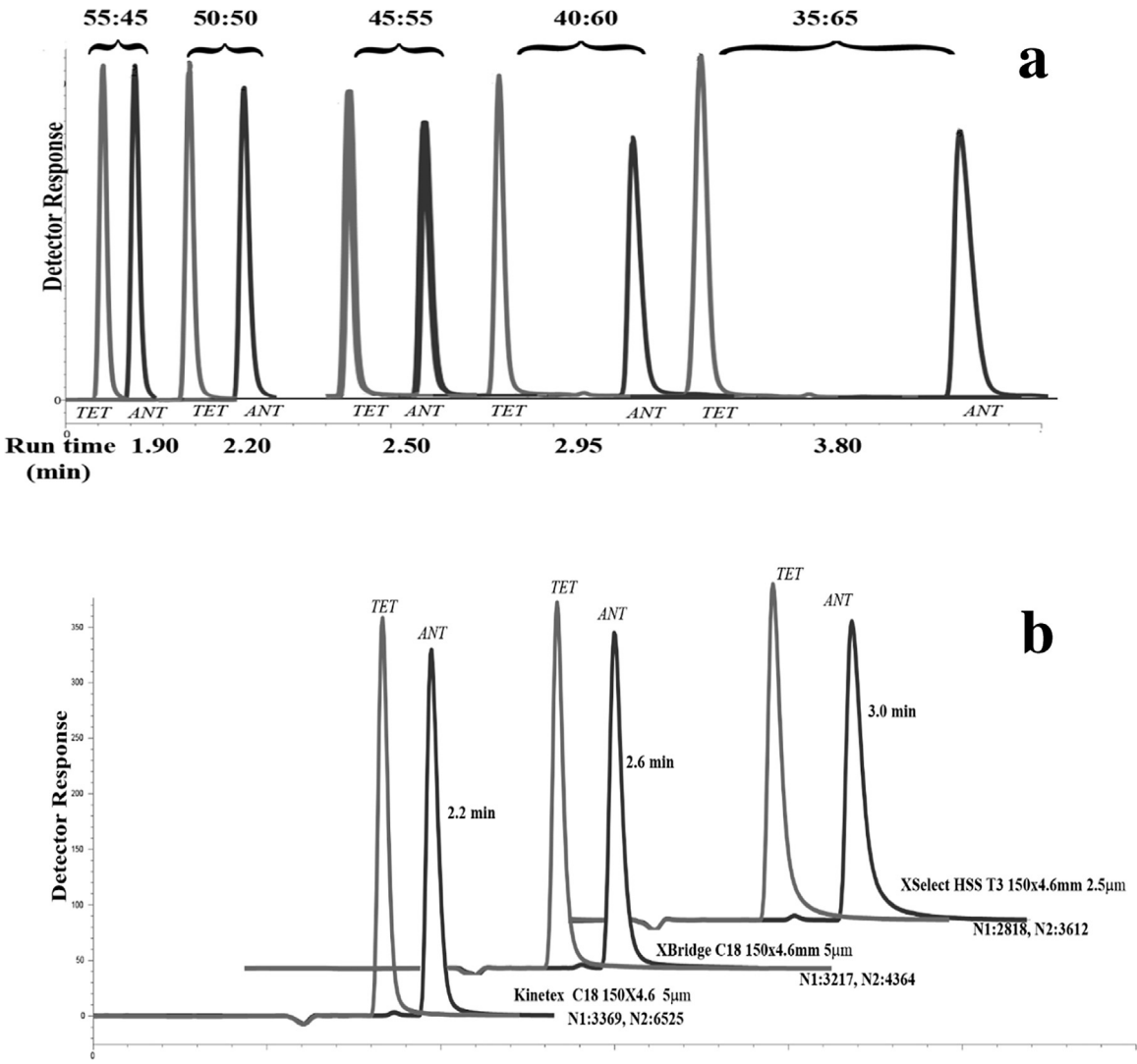


Fig. 2. Optimization of HPLC method. (a) Optimization of mobile phase, column: Kinetex C18 (150 × 4.6 mm; 5 μm), pH of the mobile phase adjusted to 3.0, 25 °C temperature with the flow rate of 1 mL min^{-1}, (b) Choice of column, pH of the mobile phase adjusted to 5.30, with a flow rate of 1 mL min^{-1}, composition of mobile phase 60:40 (v/v) ACN:Buffer (0.05% TFA), and temperature 25 °C.

2.4.3. Limits of detection and quantification

LOD and LOQ were calculated from the equations in which the standard deviation of response and the slope of the calibration curve were used [18].

2.4.4. Precision and accuracy

The intermediate precision was assessed by carrying out the analysis of at least three working solutions in three consecutive days for inter-day repeatability and it was evaluated by assaying at least five injections with using one concentration from the working range within the same day for intra-day under the same experimental conditions.

Accuracy is one of the main requirements of any chromatographic or capillary electrophoretic method especially when working on pharmaceuticals which is defined as the proximity of the experimental value to the true value for the real samples [17,18]. Recovery was carried out by spiking the already analyzed samples of dosage form with the known amounts of standard solutions of pharmaceutical active compounds in dosage forms.

3. Results and discussion

3.1. Optimization of CE conditions

3.1.1. Selection of wavelength

Optimization of CE conditions as well as the selection of optimum detection wavelength was based on corrected peak area (CPA) which offered the advantage of having a higher reproducibility than would be obtained using peak area or peak height. Absorption of ANT and TET was scanned in the range of 192–600 nm and a 3D absorption spectrum (absorption-migration time-wavelength) was recorded. It was found that both analytes absorbed in the range of 192–200 nm with absorption maxima being at 192 nm.

3.1.2. Type of buffer and its concentration

Acidic, neutral and basic buffers (i.e., acetate pH 4.6, phosphate pH 7.0 and borate buffer at pH 9.2) were evaluated to achieve optimum efficiency. Although pH of the background electrolyte (BGE) had no noticeable effect on resolution (data not shown), migration time as well as peak areas decreased with increasing the pH which was thought to be due to the lower magnitude of the electroosmotic flow (EOF) inside the capillary under acidic conditions.

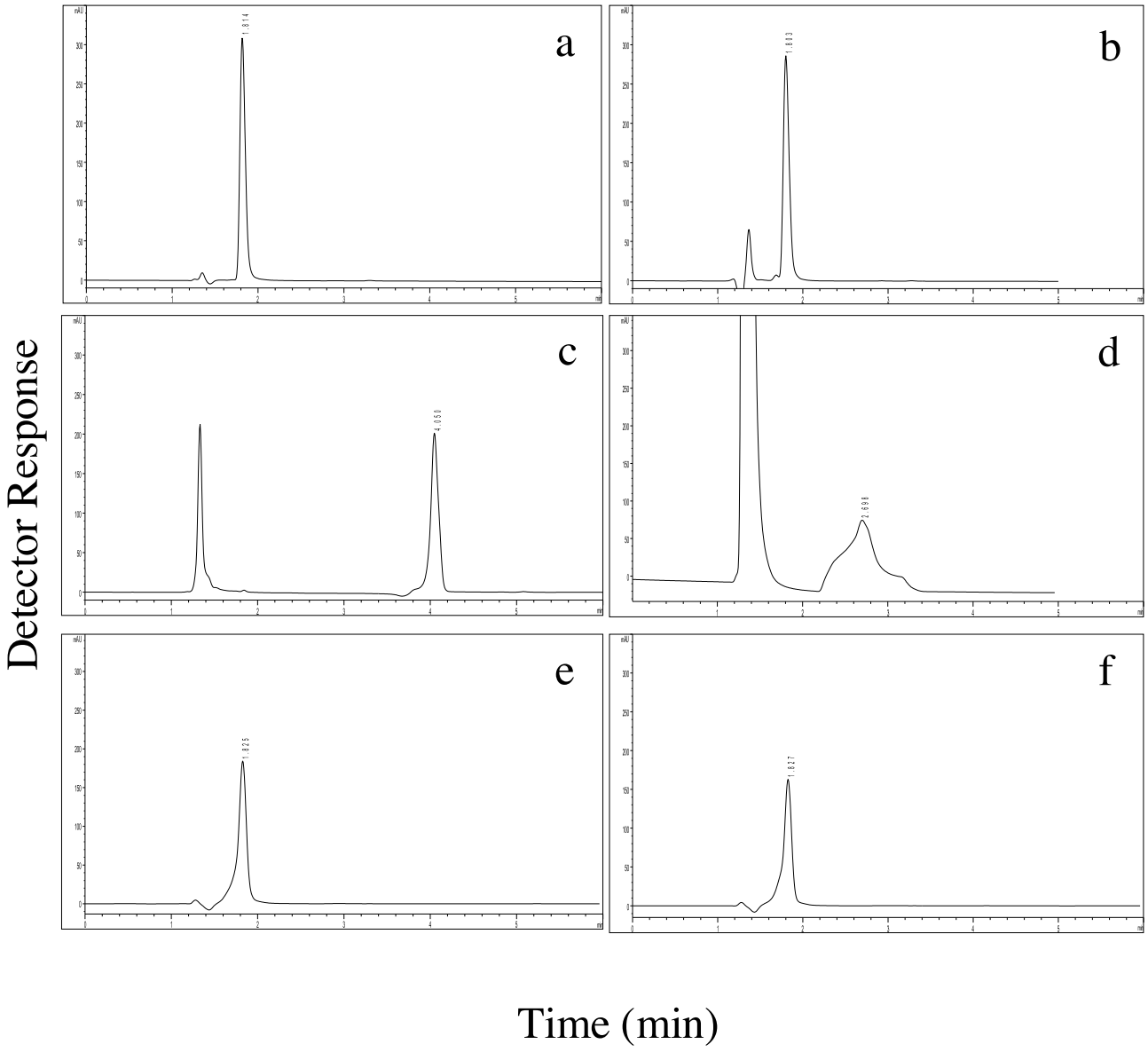


Fig. 3. Typical HPLC chromatograms of pure bulk sample of 50 μg mL^{-1} TET (a) and mild stressed conditions of samples: in 0.1 M HCl for 60 min (b); in 0.1 M NaOH for 60 min (c); in 3% H_2O_2 for 60 min (d); under UV light at 254 nm for 6 h (e); and at 100 °C for 6 h (f).

Thus, the highest CPA was achieved using acetate buffer at pH 4.6 (Fig. 1a). Despite this, phosphate buffer at pH 7.0 was preferred as a compromise between peak area and migration time. The effect of buffer concentration on CPA was studied in the range of 5–50 mM. It was observed that the highest stability was obtained within the range of 10–25 mM above which caused a high current and was thus avoided. There was no considerable effect of buffer concentration on migration time within the whole range (i.e., 5–50 mM). Above 20 mM peak area started to decrease which eventually decreased CPA (Fig. 1b). Thus, 20 mM phosphate buffer at pH 7.0 was used throughout the experiments.

3.1.3. Organic modifier

Adding a small amount of an organic solvent (modifier) such as methanol, ethanol or acetonitrile to the BGE is common in CE to improve resolution. In this study, their effect was studied with no modifier added to the BGE as well as in the range of 5–15% (v/v). It was noticed that with all of these solvents, the resolution and the peak areas were almost the same. Thus, CPA decreased with increasing the organic percentage (Fig. 1c). Hence, no modifier was used in this study.

3.1.4. Separation voltage and temperature

Separation voltage was investigated in the range of 18–27 kV. Increasing the separation voltage within this range decreased both the migration time and peak areas. Calculating CPA, however, revealed that it increased gradually up to 22 kV after which it started to decrease (Fig. 1d). Thus, 22 kV was applied thereafter.

Capillary temperature was tested over the range of 12–30 °C. CPA increased gradually up to 25 °C and then started to decrease. Increasing the separation temperature decreased both the migration time and peak areas. It was seen that CPA increased rapidly up to 25 °C and then started to decrease afterwards (Fig. 1e). Hence, this value was considered optimum for further experiments.

3.1.5. Injection time

The effect of injection time was investigated by applying a pressure of 50 mbar for 5–17 s. Although, increasing the injection time

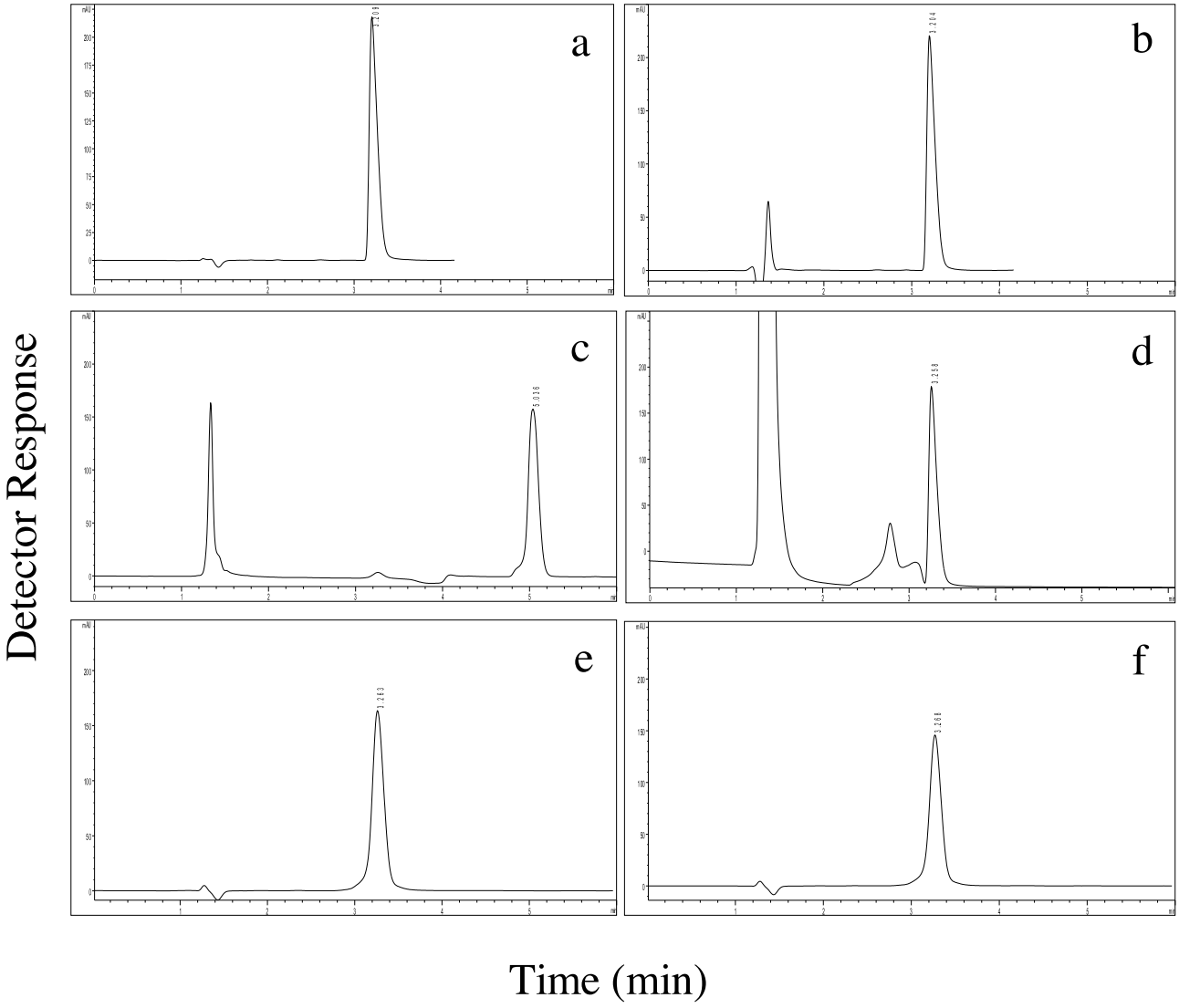


Fig. 4. Typical HPLC chromatograms of pure bulk sample of 50 μg mL^{-1} ANT (a) and mild stressed conditions of samples: in 0.1 M HCl for 60 min (b); in 0.1 M NaOH for 60 min (c); in 3% H_2O_2 for 60 min (d); (e) under UV light at 254 nm for 6 h; and at 100 °C for 6 h (f).

within this range had no noticeable effect on migration time, it eventually increased peak areas and hence CPA (Fig. 1f). Despite the fact that even at a 17-s injection time a good resolution between the peaks was still observed, higher injection times were not applied due to start of deterioration of peak symmetry. Hence, an injection time of 17 s was considered optimum.

Under the electrophoretic conditions were all optimized, the two analytes were separated and simultaneously determined in the commercial pharmaceutical preparations.

3.2. Optimization of HPLC conditions

To obtain the best HPLC conditions, step by step optimization was adopted. The point of an HPLC method development is to achieve sufficient resolution of the target analytes from all other excipients, interferences and matrix effect within a short analysis time and to obtain suitable peak symmetry with acceptable efficiency [15,19]. Different new generation stationary phases which were packed as fully porous and core-shell materials were primarily tested. Using the same isocratic conditions, an efficient separation was obtained using Kinetex C18 (150 mm × 4.6 mm i.d., 5 μm) as the analytical column. Fig. 2a demonstrates that when core-shell particles were used, sharper peaks were obtained when comparing with other columns that have traditional fully porous particles which provided much more efficient analysis in addition of reduced analysis time. Poor separation was observed with X Select column which not only increased the analysis time but also produced back pressure that was almost three times that of the other. X Bridge can also be chosen for the simultaneous determination of ANT and TET but after optimizing all conditions, the elution times with this column were longer than those obtained with the core-shell packed Kinetex column.

The effect of different organic solvents with varied compositions in the mobile phase was evaluated with different buffers. For this reason, acetonitrile and methanol were investigated as the organic solvents. Because of the narrow and sharp peak shapes obtained with acetonitrile, this solvent was selected. The effect of acetonitrile composition in the mobile phase was tested in the range of 35–55% and the results are shown in Fig. 2a. pH of the mobile phase is another remarkable point for the method development. Because of the sharper and efficient signals, TFA (0.05%) was added to the buffer and the pH was adjusted to 3.0 with 5 M NaOH. Temperature of the column oven was adjusted and studied within the range of 25–40 °C. No remarkable effect of column temperature was

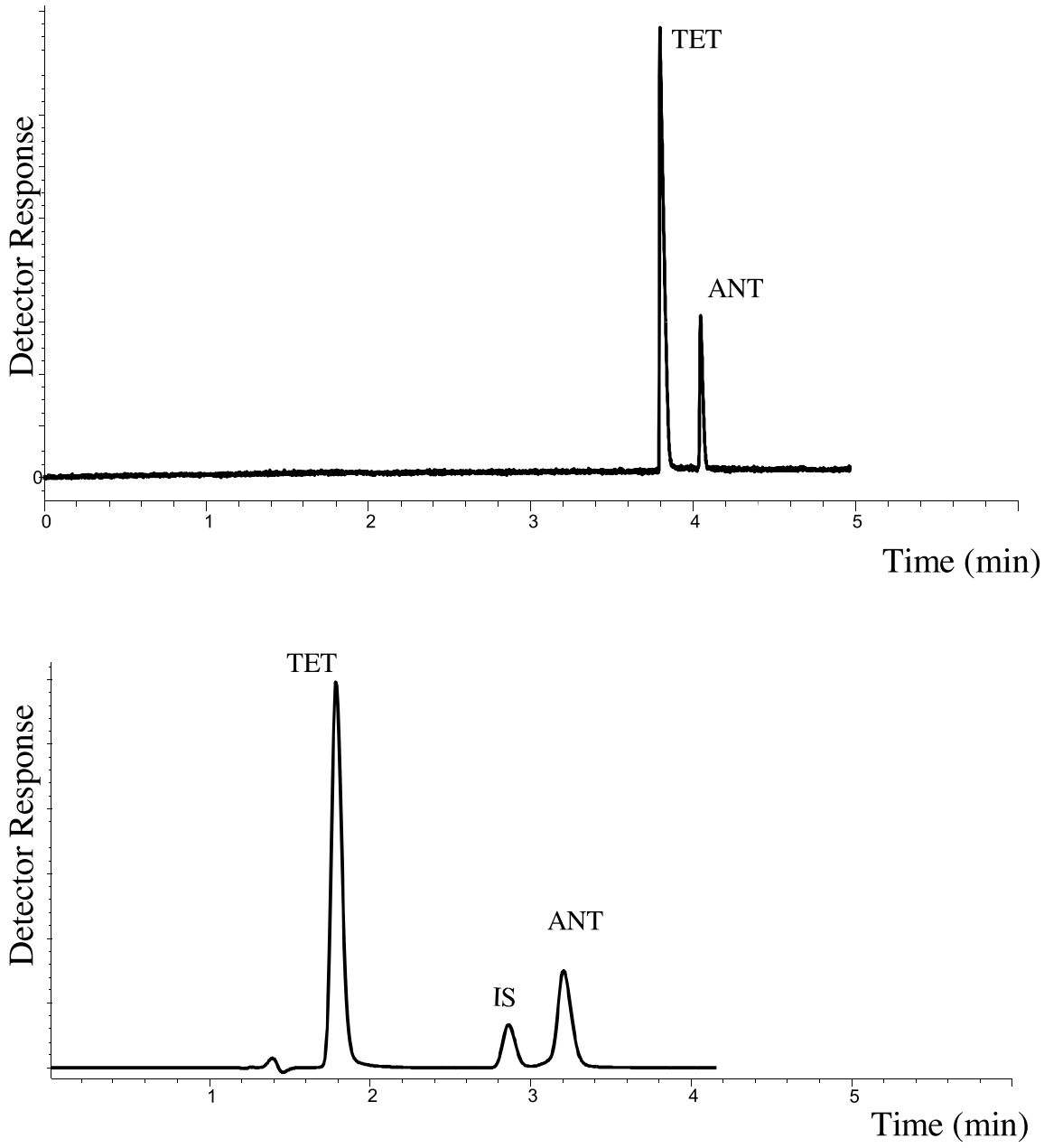


Fig. 5. Representative electropherogram (a) and chromatogram (b) of unspiked ophthalmic solution sample. 100 μg mL^{-1} TET and 30 μg mL^{-1} ANT respectively. 2 μg mL^{-1} IS was used for HPLC analysis.

observed. Therefore, 25 °C was chosen as the optimum temperature. The system suitability test results, evaluated according to the ICH, showed that the developed method was suitable for the simultaneous determination and quantification of ANT and TET. Under optimized conditions, retention times obtained were 1.785, 2.819 and 3.165 min for TET, IS and ANT, respectively (Table 1).

3.3. Analytical performance and validation

For presenting the stability indicating capability of the developed HPLC method, forced degradation studies were performed. The chromatograms are shown in Figs. 3 and 4 and degradation percentages were tabulated after each treatment as shown in Table 2.

The stock solutions of the compounds were diluted with HCl, NaOH and H_2O_2 to 50 μg mL^{-1} and waited for 1 h. As a mild condition there was very few amount of TET degraded (2.57%) (Fig. 3) with 0.1 M HCl and no degradation observed on ANT (Fig. 4). When applying drastic conditions (e.g., 1 M HCl), 3.37% of TET and 1.21% ANT degraded. In contrast, basic hydrolysis was more effective than the acidic one. As a result of mild alkaline hydrolysis, 8.43% ANT and 5.38% TET degraded after 1 h of treatment. On the other hand, 1 M of NaOH degraded TET completely and 80% of ANT. The addition of 3% H_2O_2 did not show any effect on the analytes but peak broadening of TET was observed. After treatment with 30% H_2O_2, the results were found as 13.42% and 5.24 for TET and ANT, respectively. Solid forms of both compounds were kept under UV light at 254 nm for photolytic and at 100 °C in an oven for thermal degradation. No degradation was observed on the short term (6 h) but after 24 h, the results with photolytic degradation were found as 27.76% and 10.39% and the result of thermal degradation were found as 17.27% and 35.50% for TET and ANT, respectively. As a result of these

Table 2
Results of hydrolytic, oxidizing, thermal and photolytic stress conditions.

Stress conditions		% Degradation of TET	% Degradation of ANT
Mild conditions	HCl (0.1 M)	2.57	ND
	NaOH (0.1 M)	8.43	5.38
	H_2O_2 (3%)	ND[a]	0.49
	6 h UV	ND	ND
	6 h 100 °C	ND	ND
Drastic conditions	HCl (1 M)	3.37	1.21
	NaOH (1 M)	100	80.63
	H_2O_2 (30%)	13.42	5.24
	24 h UV	27.76	10.39
	24 h 100 °C	17.27	35.50

Each value is the mean of five experiments. ND: No degradation.
[a] Peak shape getting broader.

Table 3
Regression data of the calibration graphs for quantitative determination of TET and ANT by HPLC and CE.

Compounds	HPLC		CE	
	TET	ANT	TET	ANT
Linearity range ($\mu g\,mL^{-1}$)	0.5–200	0.5–200	2.3–100	5.3–100
Slope	0.155	0.171	6.060	4.830
Intercept	0.023	0.038	−1.130	−7.440
Correlation coefficient	0.999	0.999	0.999	0.998
SE of slope	1.16×10^{-3}	6.63×10^{-4}	0.050	0.080
SE of intercept	1.05×10^{-1}	6.01×10^{-2}	1.130	2.420
Limit of detection ($\mu g\,mL^{-1}$)	0.068	0.078	0.70	1.60
Limit of quantification ($\mu g\,mL^{-1}$)	0.206	0.238	2.30	5.30
Within-day precision[a] (RSD%)	0.426	0.388	2.10	1.80
Between-day precision[a] (RSD%)	0.918	0.517	2.80	1.90

[a] Each value is the mean of five experiments.

Table 4
Assay results and mean recovery studies of TET and ANT in pharmaceutical dosage forms.

Compounds	HPLC		CE	
	TET	ANT	TET	ANT
Labeled claim ($mg\,mL^{-1}$)	0.500	0.150	0.500	0.150
Amount found ($mg\,mL^{-1}$)[a]	0.484	0.152	0.489	0.147
RSD (%)[a]	1.029	0.390	97.80	98.00
Bias (%)	3.200	−1.333	2.20	2.00
Added ($mg\,mL^{-1}$)	0.250	0.075	0.250	0.075
Found ($mg\,mL^{-1}$)[a]	0.254	0.077	0.246	0.073
Recovery (%)	101.565	102.870	98.20	97.90
%RSD of recovery[a]	0.246	0.282	1.90	2.15
Bias (%)	−1.565	−2.870	1.80	2.10

[a] Each value is the mean of five experiments.

experiments, the developed HPLC method was found as specific for the analyzed APIs.

Standard calibration graphs were constructed as mentioned above. Under optimized conditions, LODs were found as 1.6 and 0.7 $\mu g\,mL^{-1}$ by CE and 0.068 and 0.078 $\mu g\,mL^{-1}$ for TET and ANT by HPLC, respectively (Table 3). LOQs were calculated as 5.3 and 2.3 $\mu g\,mL^{-1}$ with CE and 0.206 and 0.238 $\mu g\,mL^{-1}$ for ANT and TET, respectively. The response was linear over the concentration ranges of 2.3–100, 5.3–100 with CE and 0.5–200 $\mu g\,mL^{-1}$ with HPLC for ANT and TET, respectively, with R^2 not lower than 0.998 with both methods. Reproducibility was evaluated in terms of intraday and interday precision, by injecting the standards at five concentration levels in the same day and in three consecutive days, respectively. An acceptable precision was obtained in both cases RSD% values below 2.8% for interday and 2.1% for intraday experiments with CE and 0.426% for interday and 0.918% for intraday assays with HPLC.

3.4. Analysis of pharmaceutical dosage forms

In order to evaluate the applicability, recovery and possible matrix effect of the proposed CE method, the commercial ophthalmic solution (Allergoconjunct®) with reported concentration of 0.15 $mg\,mL^{-1}$ ANT and 0.50 $mg\,mL^{-1}$ TET was examined. It was ensured that the removal of the excipients with an extraction step before analysis was unnecessary. As indicated by Table 4, the recovery results were found in the range between 97.90–102.87% for both methods. It was concluded that the proposed methods were sufficiently accurate and precise for the pharmaceutical dosage form of TET and ANT. The proposed methods were compared statistically. Both methods showed similar accuracy and precision. A statistical comparison was performed on data obtained from both techniques. Student's t- and F test revealed no statistically significant difference between methods with regard to accuracy and precision (Table 4) [16–19]. Typical chromatogram and electropherogram of ophthalmic solution sample are shown in Fig. 5a and b. The analytes were baseline separated in less than 4.5 min with both techniques.

4. Conclusion

Neither CE nor stability-indicating HPLC methods are found in the literature for the simultaneous determination of ANT and TET from pharmaceutical dosage forms. For this reason, CE and stability-indicating HPLC methods were fully validated according to the ICH guidelines and were presented for the determination of ANT and TET in ophthalmic formulations which offers numerous advantages, such as rapidity, use of minimum amounts of organic solvents, simplicity, low cost, ease of operation, and high selectivity. Good recoveries, high reproducibility and interference-free electropherograms and chromatograms were also achieved. Even CE is studied as a comparison method, it showed better peak capacity and efficiency. Furthermore, the conditioning time of CE showed an advantage over HPLC.

The proposed methods present a step by step optimization procedure. In this study, new generation stationary phases were compared for the first time with the new column packing materials especially the core-shell particles. As a result of this study, the proposed methods are suitable for quality control laboratories, where economy and time are essential. High percentage of recovery results showed that the proposed methods were free from interferences of commonly used excipients and additives in the formulations.

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