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Article

Simultaneous determination of paracetamol, dextromethorphan, chlorpheniramine, pseudoephedrine and major impurities of paracetamol and pseudoephedrine by using capillary electrophoresis

Beytul Yener[1], Cem Erkmen[2,3], Bengi Uslu[2], Nilgun Gunden Goger[1*]

[1]Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, 06330, Ankara, Turkey, [2]Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara, Turkey, [3]Ankara University, The Graduate School of Health Sciences, 06110, Ankara, Turkey

A capillary electrophoresis method was developed for the first time and optimized for the determination of paracetamol, pseudoephedrine, dextromethorphan, chlorpheniramine, 4-aminophenol and ephedrine in tablet formulation. Optimum electrophoretic conditions were achieved by using a background electrolyte of 75 mmol L^{-1} sodium borate buffer at pH 8.0, a capillary temperature of 30°C, a separation voltage of 30 kV and a pressure injection of the sample at 50 mbar for 10 s. Calibration graphs showed a good linearity with a coefficient of determination (R^2) of at least 0.999 for all compounds. Intraday and interday precision (expressed as relative standard deviation (RSD) %) were lower than 1.39% for capillary electrophoresis method. The developed method was demonstrated to be simple and rapid for the determination of paracetamol, pseudoephedrine, dextromethorphan, chlorpheniramine, 4-aminophenol and ephedrine in tablet formulation providing recoveries in the range between 99.62 and 100.57% for all analytes.

Keywords: Capillary electrophoresis. Chlorpheniramine. Dextromethorphan. Paracetamol. Pseudoephedrine.

INTRODUCTION

Paracetamol (PAR) (Figure 1a) is an acetanilide derivative. It is known as chemically 4-hydroxy acetanilide having analgesic, antipyretic and anti-inflammatory action (Devi *et al*., 2013). Dextromethorphan (DEX) (Figure1b) (3-methoxy-17-methylmorphinan) is a dextrorotatory optical isomer of levomethorphan, a typical morphine like opioid. DEX is an effective cough suppressant with negligible side effects at antitussive dose range. However, DEX produces a very complex pharmacological profile at very high doses (Tran *et al*., 2016) Chlorpheniramine (CHL) (Figure 1c), is a powerful first-generation alkyl amine antihistamine that antagonises the H1-receptors. Its chemical name is 3-(p-chlorophenyl)-3-(2-pyridyl)-N,N-dimethyl propylamine. It is widely used for symptomatic relief of the common cold and allergic rhinitis with weak sedative properties (Jain, Sharma, 2015). Pseudoephedrine (PSE) (Figure 1d) is (1S,2S)-2-(methylamino)-1 phenylpropan-1-ol hydrochloride. It is a stereoisomer of ephedrine and has similar action. PSE is orally used for the symptomatic relief of nasal congestion. Also, it is commonly used in combination with other ingredients in pharmaceutical dosage forms intended for the relief of cough and cold symptoms (Abdelwahab, Abdelaleem, 2017).

During the synthesis and/or storage of PAR, it can be partly decomposed to 4-aminophenol (4-AP) (Figure 1e), which is the primary impurity of PAR in pharmaceutical formulations. The presence of 4-AP in PAR leads to toxicity, which includes the nephrotoxicity and hepatotoxicity of patients. (Nemakal *et al.,* 2019)

***Correspondence:** N. G. Goger. Gazi University. Faculty of Pharmacy. Department of Analytical Chemistry. 06330, Ankara, Turkey. Phone: +90 3122023104. Fax: +90 312 4333419. E-mail: ngoger@gazi.edu.tr. ORCID iD: https://orcid.org/0000-0002-7376-9879

Ephedrine (EPH), 2-methylamino-1-phenylpropan-1-ol, (Figure 1f) is a medication extracted from a plant called *Ephedra sinica* that acts as a sympathomimetic stimulant on central nervous system to prevent low blood pressure in cardiovascular diseases and hypotension caused by anesthesia. Also, EPH is the major impurity of PSE. (Baharfar *et al.,* 2017)

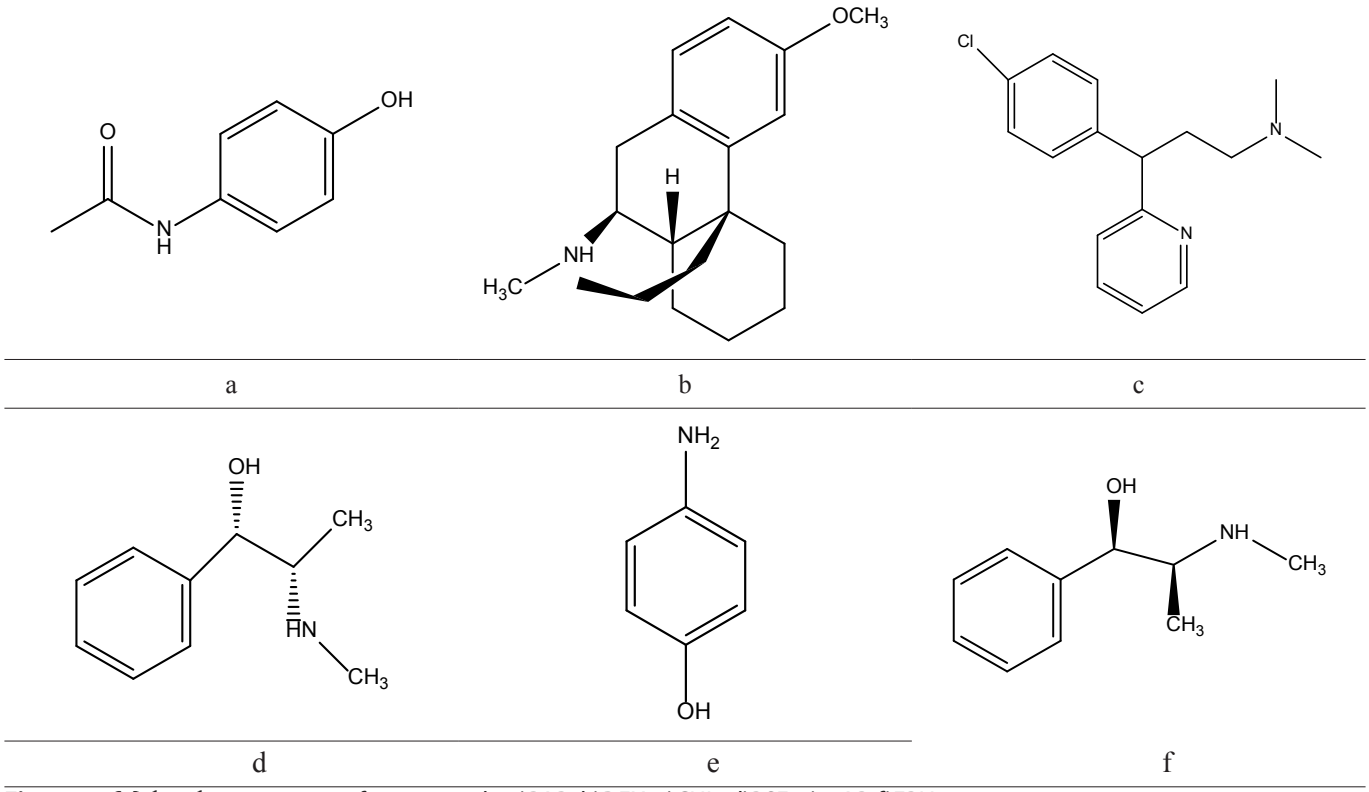

Figure 1 - Molecular structures of compounds, a)PAR, b)DEX, c)CHL, d)PSE, e)4-AP, f)EPH.

Literature studies have shown that there are different analytical methods for determination of binary or ternary mixtures of PAR, DEX, CHL and PSE in pharmaceutical formulations, cold syrup formulation and or biological matrix. Fluorescence spectrophotometric (Azmi *et al*., 2017), spectrophotometric and chemometric (El deen Sayed *et al*., 2013; Shrestha, Pradhananga, 1970), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Hu *et al*., 2011; Celma *et al*., 2000, Li *et al.,* 2009), reverse phase high performance liquid chromatographic (RP HPLC) (Hood, Cheung, 2003; Abdelwahab, Abdelaleem, 2017; Dewani *et al*., 2014; Palabiyik, Onur, 2007), liquid chromatography/quadrupole-time-of-flight mass spectrometry (LC/Q- TOF-MS) (Thurman, Ferrer, 2012), liquid chromatography method with fluorimetric detection (Afshar, Rouini, Amini, 2004), capillary electrophoretic (CE) (Kristensen, 1998), raman spectroscopy (Ali *et al*., 2019), electrochemical method (Tyszczuk-Rotko, Jaworska, Jedruchniewicz, 2019), high performance liquid chromatography coupled to diode array detection (HPLC-DAD) (Santos *et al*., 2013), HPLC–MS/MS (Hewavitharana *et al*., 2008), capillary zone electrophoresis (CZE) (Capella- Peiro *et al*., 2006), high performance thin layer chromatography (HPTLC) (Makhija, Vavia, 2001), derivative spectrophotometry and ratio spectra derivative spectrophotometry (Onur *et al*., 2000), nonaqueous CE (Dong *et al*., 2005), and normal-phase LC (Al-Rimawi, 2010) methods have been

reported in literature for the determination of PAR, DEX, CHL and PSE.

A combination of PAR, PSE, DEX and CHL are used in pharmaceutical dosage forms for reducing symptoms associated with the common cold. PAR has analgesic and antipyretic effects. PSE acts as a decongestant. DEX is used as an antitussive agent. Also, CHL is used as an antihistamine. Therefore, it is important to carry out simultaneous determination of these compounds. In the literature, there is only one method in which these compounds can be analyzed simultaneously (Al-Rimawi, 2010).

Nowadays, miniaturized separation techniques such as CE, capillary electrochromatography (CEC) and micro/capillary/nano liquid chromatography (micro LC/CLC/nano-LC) have become greatly popular in pharmaceutical analysis. These separation methods are increasingly utilized in all processes of drug discovery as well as quality control of pharmaceutical preparations (Aturki *et al*., 2014).

The aim of this work is to develop a rapid and simple CE method for the simultaneous determination of PAR, 4-AP, PSE, EPH, DEX and CHL. Furthermore, the another aim of the study is to prove the accuracy of the method by applying the developed method to the pharmaceutical dosage form.

MATERIAL AND METHODS

Material and chemicals

All experiments were carried out on an Capillary Electrophoresis system (Hewlett Packard Agilent 3D-CE, UV diode array detector, computer controlled, Agilent Technologies, Waldbronn, Germany). pH measurements were performed with Orion 720 A+ potentiometer (Termo Fischer Scientific, New Hampshire, USA). Paracetamol, chlorpheniramine, pseudoephedrine and dextromethorphan were obtained from Kocak Farma (Istanbul, Turkey). 4-aminophenol, ephedrine, sodium borate, sodium hydroxide (NaOH), hydrochloric acid (HCl; 37 % (w/w)) and acetonitrile (ACN; gradient grade for liquid chromatography) were obtained from Merck KGaA (Darmstadt, Germany). Chromatographic grade water with conductivity lower than 0.05 μS cm^{-1} was obtained through a Millipore direct-Q 3UV ® water purification system (Millipore, Bedford, MA, USA). Gripamol® tablet as a pharmaceutical dosage form was obtained from Kocak Farma (Istanbul, Turkey).

Methods

Preparation of solutions

The powdered sodium borate was weighed at 5.42 g and placed in a 100.0 mL flask and dissolved. Stock solution (150 mmol L^{-1}) was stored at 4°C in the refrigerator. 75 mmol L^{-1} borate buffer solution was prepared from the stock sodium borate buffer solution and adjusted to pH 8.0. The background electrolyte (BGE) as a working buffer solution was prepared by adding 15% acetonitrile to 75 mmol L^{-1} borate buffer solution (pH 8.0).

The pure PAR in powder form was weighed to 10.0 mg precisely and placed in a 10.0 mL flask. This stock solution was waited for about 5 minutes at 40°C in an ultrasonic bath. Standard PAR solutions were prepared from this stock solution by diluting with BGE solution. Standard solutions of 4-AP, PSE, EPH, DEX and CHL were prepared by the same method. The stock solution of 4-AP was freshly prepared prior to each experiments because of its rapid deterioration and discoloration.

Twenty tablets of Gripamol® containing PAR, PSE, DEX and CHL were precisely weighed and powdered in a mortar. The tablet powder, which is equal to the weight of a tablet, was weighed precisely and taken to a 100 mL flask. 6.0 mg of EPH and 6.0 mg of 4-AP were added to this flask. Finally, the mixture was completed to 100.0 mL. 10.0 mL of this solution was centrifuged at 5000 rpm for 10 minutes. 2.5 mL of the clear solution was taken by automatic pipette and this solution was completed with 10.0 mL of BGE solution; A solution containing 812.5 μg mL^{-1} PAR, 5 μg mL^{-1} CHL, 75 μg mL^{-1} pPSE, 37.5 μg mL^{-1} DEX and 15 μg mL^{-1} EPH and 15 μg mL^{-1} 4-AP was obtained.

Optimization of method

Optimum conditions were investigated by changing the parameters of BGE solution (type of solution, pH

and concentration of BGE solution, and effect of organic solvent) and other parameters (capillary diameter, injection time, applied voltage, temperature and detector wavelength) for the simultaneous analysis of PAR, 4-AP, PSE, EPH, DEX and CHL.

Calibration curves

Calibration solutions containing 50, 100, 200, 400, 800, 900 μg mL^{-1} PAR, 5, 7.5, 10, 15, 20 μg mL^{-1} 4-AP, 25, 50, 75, 100, 120 μg mL^{-1} PSE, 2, 3, 5, 7.5, 10 μg mL^{-1} CHL, 5, 7.5, 10, 15, 20 100 μg mL^{-1} EPH and 25, 37.5, 50, 75, 100 μg mL^{-1} DEX were prepared by using BGE solution. Correlation (*r*) and determination (R and R^2) coefficients were calculated by plotting the peak areas versus analyte concentrations.

Validation studies

CE method was developed and validated according to the International Council on Harmonisation (ICH Guideline (Q2A) (R1) Validation of Analytical Procedures: Text and Methodology, 2005) guidelines with respect to specificity, linearity, accuracy, precision, etc. The limit of detection (LOD) value was found according to the concentration in which the S / N (signal / noise) ratio was 3. The limit of quantification (LOQ) value was found by measuring the value in which the S / N ratio was 10. Repeatability studies were carried out in two different parameters. Intra-day precision studies were performed on the same day by using three parallel solutions. Also, inter-day precision studies were performed on different days by using three parallel solutions. For this purpose, the mixture containing the standard compounds at the same concentration was analyzed seven times with the developed method and the RSD (relative standard deviation) values were calculated. Recovery studies were performed to determine the accuracy of the developed method. The accuracy of the method was determined by calculating the mean% recoveries of seven determination of the six compounds at 100 mg $tablet^{-1}$. For this aim, standard solutions were added to the known amounts of mixture solution contaning tablet ingredients and impurities. The recovery values were calculated using the calibration lines prepared with standard solutions from the electropherograms obtained from the injection of these solutions.

RESULTS AND DISCUSSION

Evaluation and optimization of separation conditions

Selection of the BGE solution and components

In the CE analysis, the separation, sensitivity, migration time and peak shape of the analytes are closely related to BGE solution. Therefore, it is significant to choose a proper BGE solution. Aqueous buffers are mostly used as BGE in CE. In the literatüre (Capella-Peiro *et al*., 2006; Dong *et al.,* 2005)., phosphate, acetate and borate buffers were found to be suitable for the separation of selected analytes. Phosphate, acetate and borate buffers were prepared at different pH values to determine optimum working buffer solution and sodium borate (pH 8.0) buffer was determined as BGE solution. The strongest acidic pKa values are 9.46 and 10.4 for PAR and 4-AP, respectively. Therefore, they are in neutral form at studied pH. Similarly, the strongest basic pKa values are 9.52, 9.52, 9.85 and 9.47 for EPH, PSE, DEX, and CHL, respectively. These compounds are in cationic form at studied pH. In CE studies, the change in migration time depends on the cationic, anionic or neutral form of compounds. PAR and 4-AP were in neutral form in this study, therefore, they were detected at a later time than other compounds. Moreover, EPH, PSE, DEX, and CHL were detected at a earlier time than PAR and 4-AP because of cationic forms of EPH, PSE, DEX, and CHL. When the migration times (Table SI) of the compounds were examined, it can be said that pH 8.0 is suitable for analysis.

Table SI - Parameters of system suitability tests

Parameters	DEX	EPH	CHL	PSE	4-AP	PAR
Average migration time (min)	5.83±0.03	6.05±0.08	6.53±0.05	7.21±0.01	9.23±0.05	10.79±0.08
Number of theoretical plates (N; plate/ meter)	9886	10164	11642	8766	9884	12692
Asymmetry factor	0.98	1.01	0.97	1.02	1.07	1.03
Average peak area	143.92±0.79	89.32±0.34	71.55±0.92	634.64±1.44	103.65±0.44	3436.60±6.38
Capacity factor	3.85	4.04	4.43	5.01	6.69	8.04
Resolution (Rs)	-	3.44	3.21	5.22	10.2	8.75
Selectivity (α)	-	1.05	1.10	1.14	1.33	1.21

BGE concentrations affect not only the resolution of compounds but also the sensitivity and migration time in the CE (Tang, Xu, Xu, 2019). Therefore, BGE concentration plays an significant role in the analysis. Different sodium borate concentrations on the separation of six compounds have been examined for a better separation. As it is shown in Figure 2, when the concentration of sodium borate was 75.0 mmol L^{-1}, each compounds can be efficiently separated with a well sensitivity. When the concentration of sodium borate was 25.0 mmol L^{-1} and 50.0 mmol L^{-1}some of the compounds were not well separated because of the large velocity of electroosmotic flow (EOF). When the concentration of sodium borate was increased to 100.0 mmol L^{-1}, the peak shapes became poor. The reason of this may be high electrical conductivity and Joule heat. As a result, the 75.0 mmol L^{-1} concentration of sodium borate was selected as the optimum concentration for further experiments.

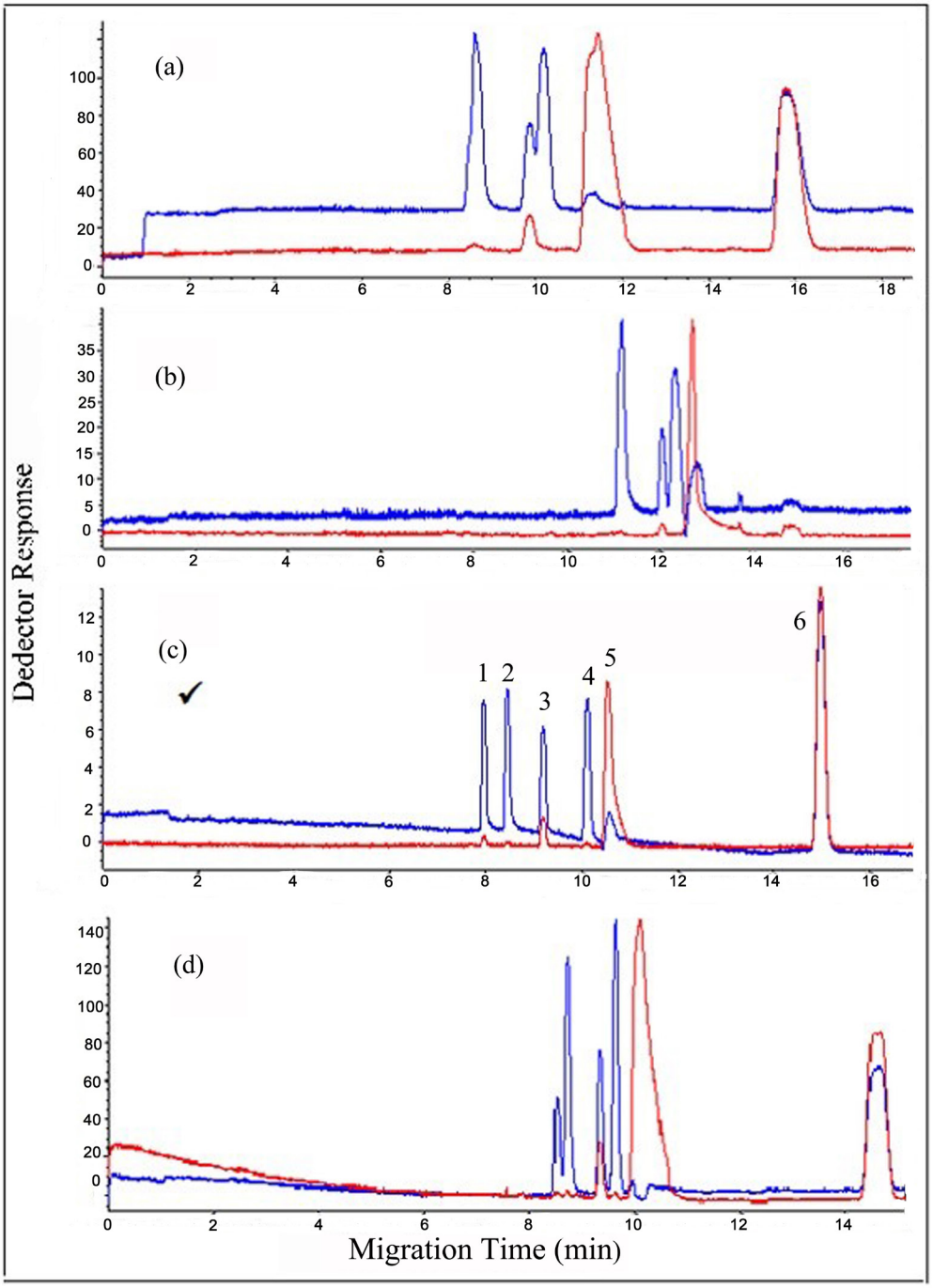

Figure 2 - Effect of buffer concentration on analysis λ=214 nm (blue line), λ=257 nm (red line), Buffer: NaBorat (pH 8), Buffer concentrations: a)25 mM b)50 mM c)75 mM d)100 mM, Conditions: Capillary diameter 50 µm, Hydrodynamic injection Pinj=50 mbar, Isolation Voltage=30 kV, T: 20ºC, Peaks: 1)DEX 2)EPH 3)CHL 4)PSE 5)4-AP 6)PAR

The addition of an organic solvent as a modifier to an aqueous buffer solution offers a number of advantages for CE analysis. The main goal of using organic solvents as an additive to the buffer electrolyte in CE is to modulate separation, selectivity, sensitivity and resolution through direct effects on the physicochemical properties of the buffer electrolyte, the capillary wall zeta potential and the analytes themselves (Yang, Boysen, Hearn, 2004). Besides, increasing the solubility of some analytes, enhancing the stability of

other analytes that may be labile in aqueous solution and reducing the interaction of hydrophobic compounds with the capillary wall.

In this study, percentage of ACN was changed from 0 to 30% (v/v). As it is shown in Figure S1, when the percentage of ACN is 15%, each compounds can be well separated with a good sensitivity. PAR, 4-AP and PSE have relatively lower logP values than other compounds. When percentage of ACN was increased, shape of these peaks improved.

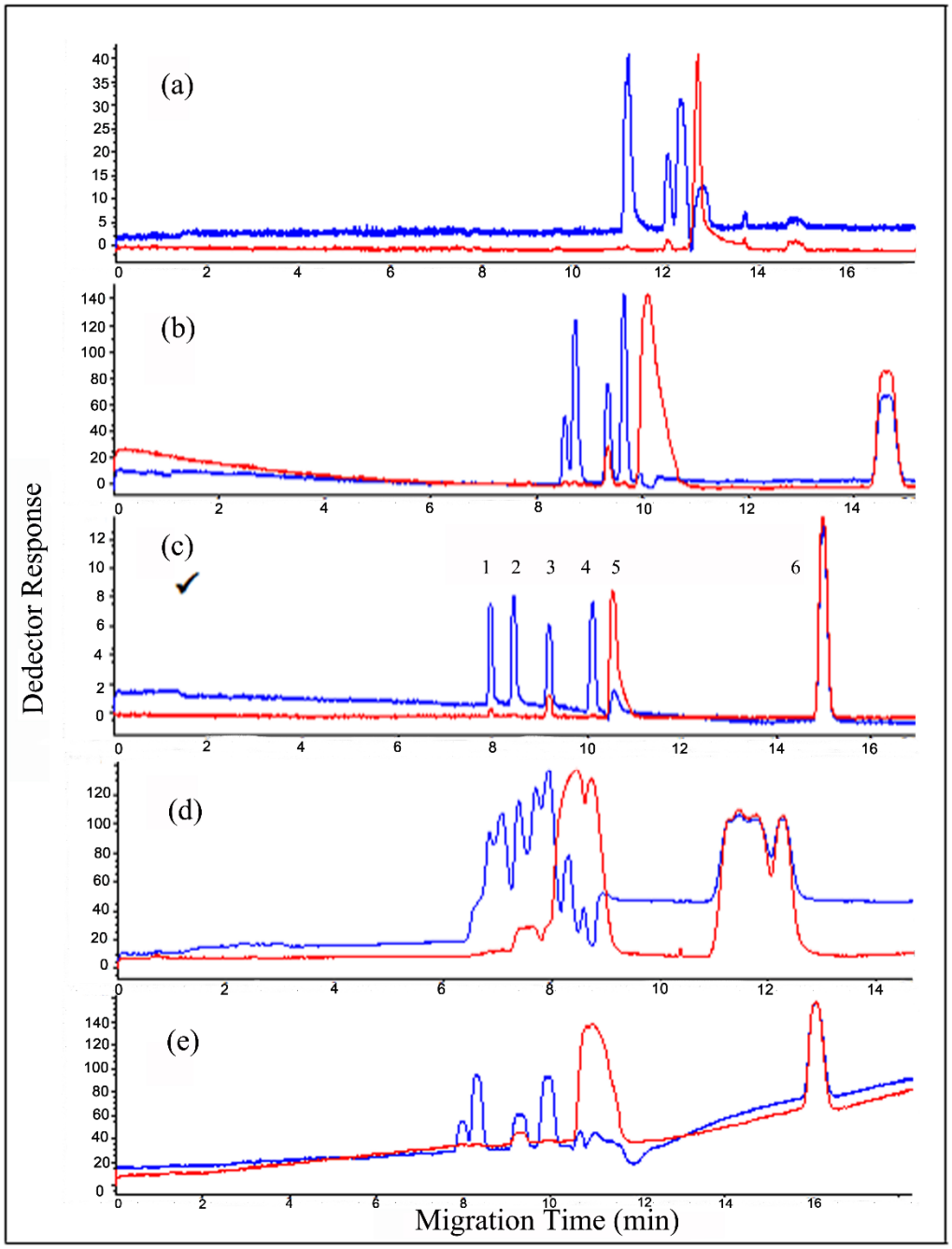

Figure S1- Effect of percentage of organic solvent on analysis λ=214 nm (blue line), λ=257 nm (red line), Percentages of acetonitrile: a)30% b)20% c)15% d)10% e)0%,

Conditions: Capillary diameter 50 µm, Hydrodynamic injection P_{inj}=50 mbar, Separation Voltage=30 kV, T: 20ºC, BGE: 75 mmol L^{-1} Sodium borate (pH: 8.0) buffer, Peaks: 1)DEX 2)EPH 3)CHL 4)PSE 5)4-AP 6)PAR

Effect of capillary column and temperature

Another important parameter in CE studies is the capillary column used. During the studies, the effect of capillary diameter was investigated and two capillaries of 50 µm and 75 µm diameter were used for this purpose. It is observed that sensitivity, peak symmetry and resolution are better with 75 µm diameter capillary as shown in Figure S2.

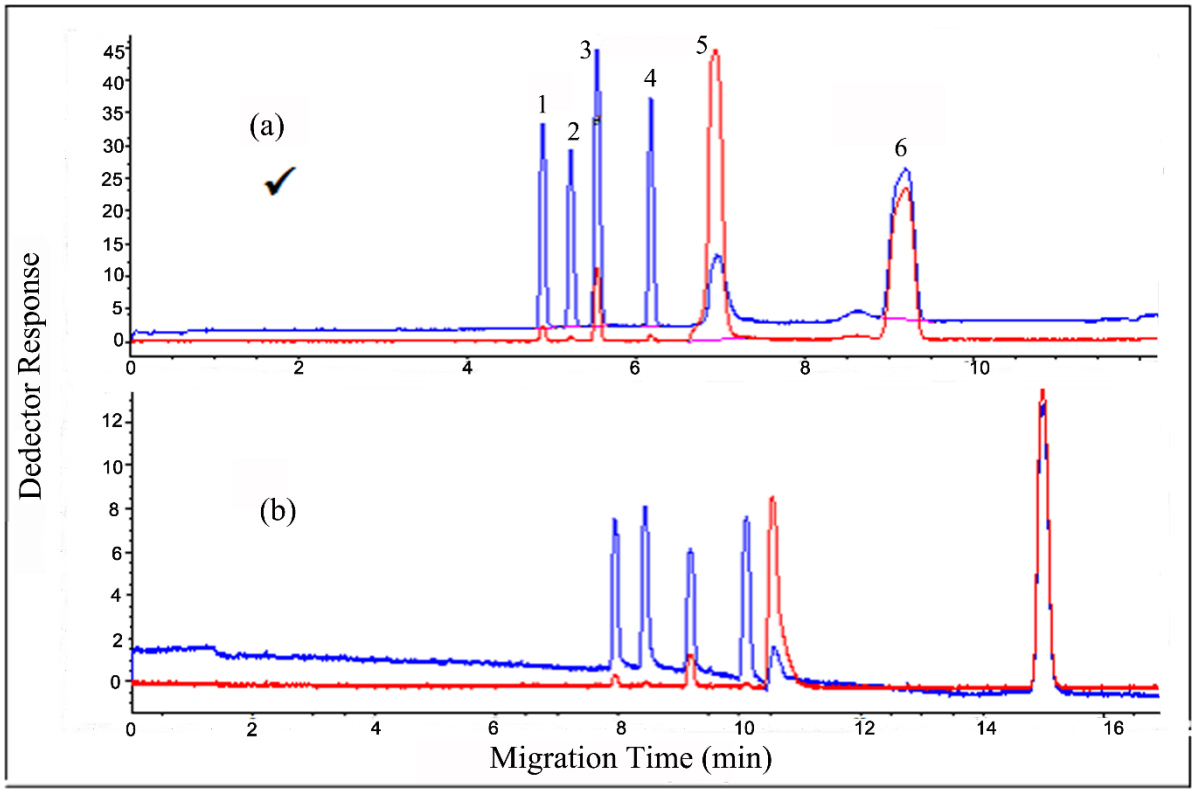

Figure S2 - Effect of capillary diameter on analysis λ=214 nm (blue line), λ=257 nm (red line), Capillary diameters: a)75 µm b)50 µm, Conditions: Hydrodynamic injection P_{inj}=50 mbar, Separation Voltage=30 kV, T: 20ºC, BGE: 75 mmol L^{-1} Sodium borate (pH: 8.0) buffer-15% ACN, Peaks: 1)DEX 2)EPH 3)CHL 4)PSE 5)4-AP 6)PAR

Capillary temperature was investigated over the range of 15-30°C. Increasing the separation temperature increased both resolution and selectivity. Also, peak shapes were improved. It was seen that 30°C is suitable for separation (Figure S3). Therefore, 30°C was considered as optimum temperature.

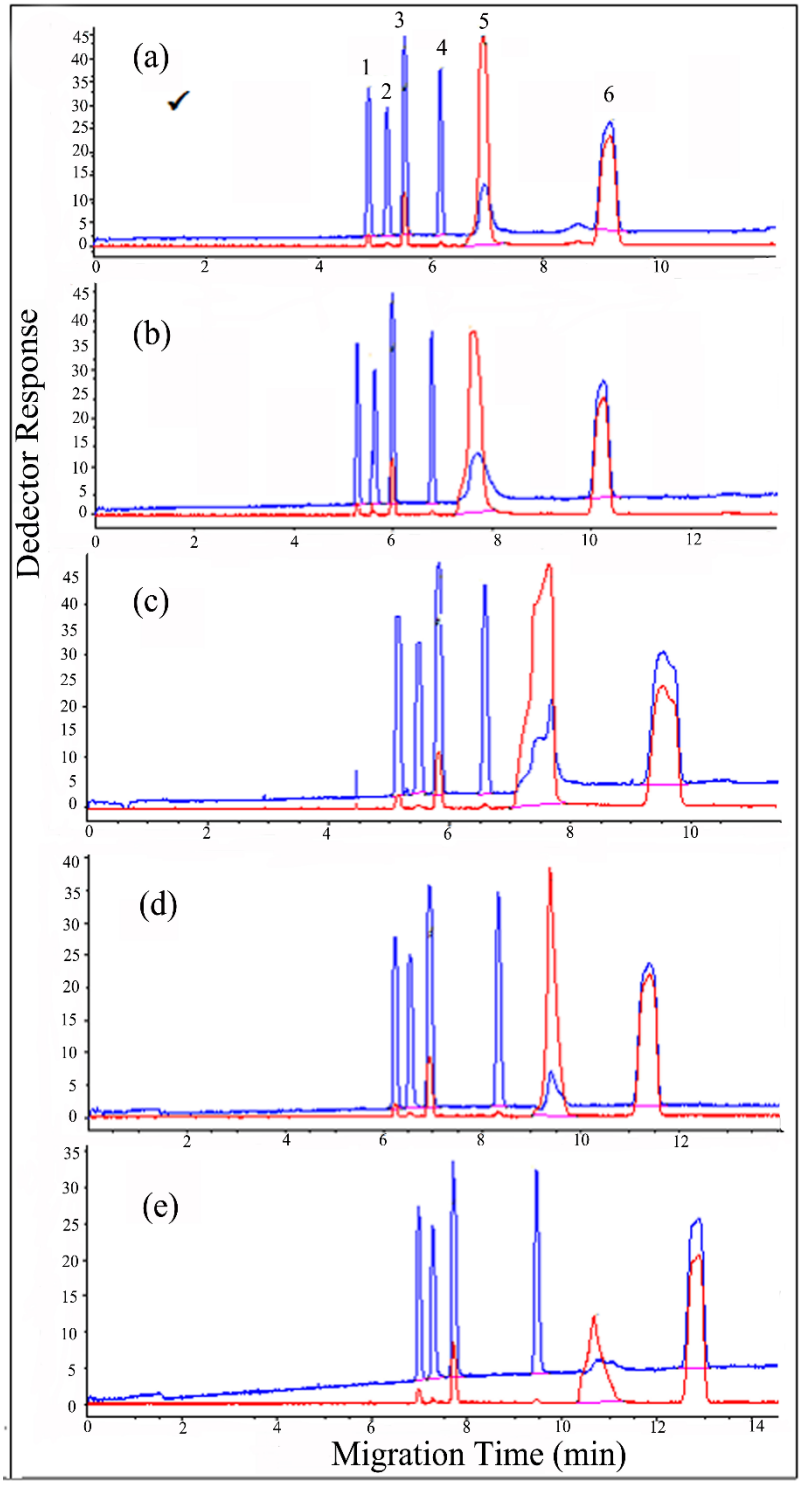

Figure S3 - Effect of temperature on analysis λ=214 nm (blue line), λ=257 nm (red line), Temperatures: a)30ºC b)25ºC c)20ºC d)15ºC e)10ºC, Conditions: Hydrodynamic injection Pinj=50 mbar, Isolation Voltage=30 kV, Capillary diameter: 75 µm, BGE: 75 mmol L-1 Sodium borate (pH: 8.0) buffer-15% ACN, Peaks: 1)DEX 2)EPH 3)CHL 4)PSE 5)4-AP 6)PAR

Effect of sample pH, selection of wavelength and injection time

pH can affect the surface charge of analytes, pH of the sample can have a great effect on the migration time. In this study, the effect of sample's pH values were examined in the range of 3.0 to 11.0. As shown in Figure S4, the peak shapes and resolution is not suitable at acidic or basic pH values. Therefore, pH 7.0 was chosen as the optimal sample's pH (in terms of sensitivity) and was used in further analysis.

Optimization of CE method conditions as well as the selection of optimum detection wavelength was based on peak area which offered the advantage of having a higher reproducibility than would be obtained using peak area or peak height.

Absorption value of analytes was scanned in the range of 190–260 nm. Then, 3D absorption spectrum (absorption-migration time-wavelength) was recorded in Figure S5. It was found that all analytes absorbed in the range of 192–200 nm and maximum absorption was choosen at 192 nm.

The effect of injection time was investigated by applying a pressure of 50 mbar for 5–20 s. However, increasing the injection time within this range had no noticeable effect on migration time. Despite the fact that well resolution between the peaks was observed at a 20-s of injection time, higher injection times were not applied due deterioration of peak symmetry. Therefore, 10 s injection time was choosen as optimum.

Optimum experimental parameters and system suitability test results, found as a result of method development studies, were presented in Table I and Table SI, respectively.

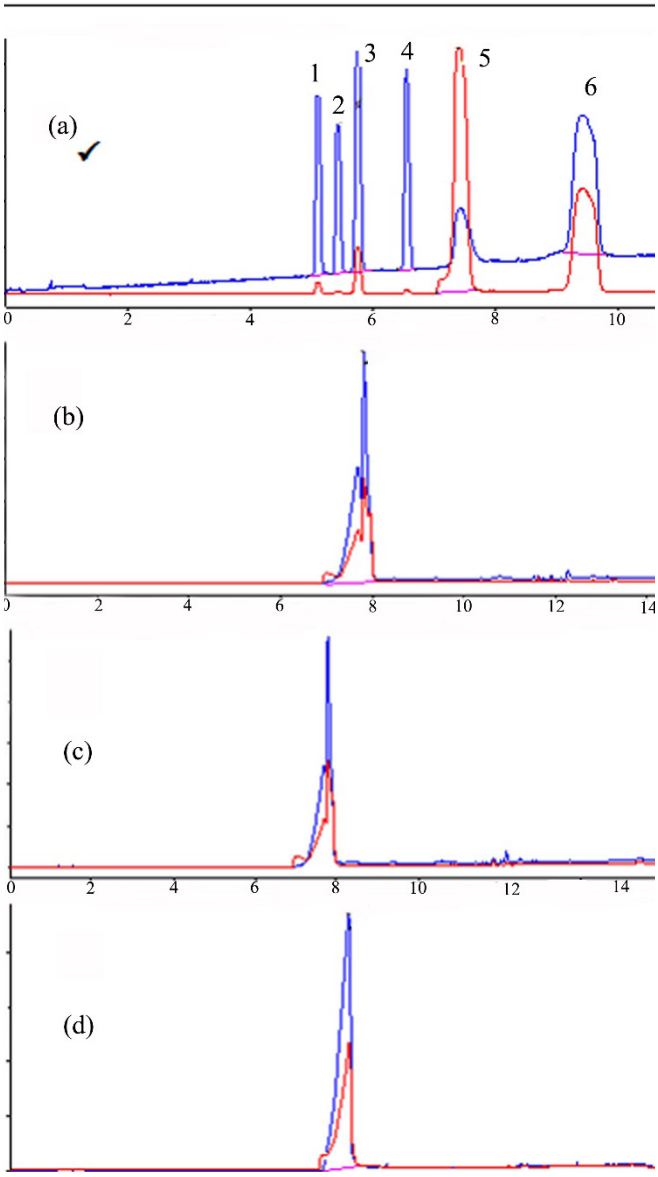

Figure S4 - Effect of sample pH on analysis, λ=214 nm (blue line), λ=257 nm (red line), a)pH 7.0 b)pH 9.0 c)pH 11.0 d) pH 5.0 e)pH 3.0, Conditions: Hydrodynamic injection P_{inj}=50 mbar, Separation Voltage=30 kV, Capillary diameter: 75 µm, BGE: 75 mmol L^{-1} Sodium borate (pH: 8.0) buffer-15% ACN, Peaks: 1)DEX 2)EPH 3)CHL 4)PSE 5)4-AP 6)PAR

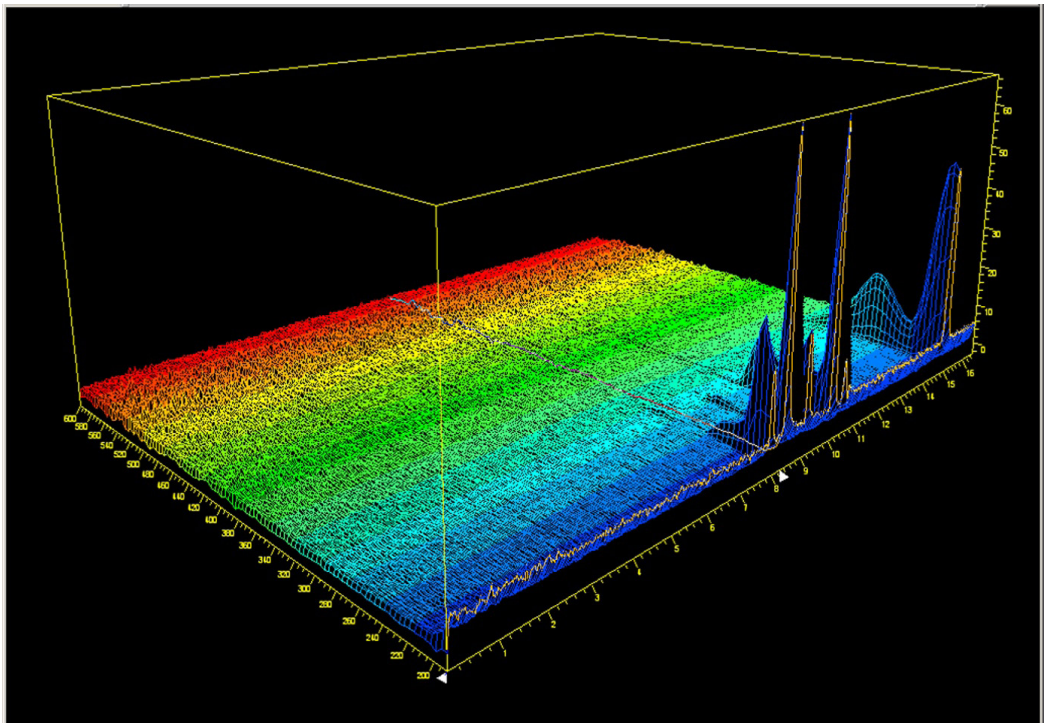

Figure S5 - 3D image of electropherogram

TABLE I - Optimization parameters

BGE solution	**75 mM sodium borate (pH: 8.0)**
Organic solvent	15%- Acetonitrile
Wavelength	192 nm
Applied voltage	30 kV
Capillary temperature	30 °C
Sample pH	7.0
Injection time	10.0 s
Diameter of capillary	75 µm

Analytical performance and validation

Under optimum conditions, different concentrations of PAR (50, 100, 200, 400, 800, 900 µg mL^{-1}) were analyzed and the calibration curve was constructed by plotting the peak height of the PAR signal observed in the electropherogram. Same measurements were carried out for 4-AP (5, 7.5, 10, 15, 20 µg mL^{-1},) PSE (25, 50, 75, 100, 120 µg mL^{-1}), CHL (2, 3, 5, 7.5, 10 µg mL^{-1}) EPH (5, 7,5, 10, 15, 20 µg mL^{-1}) and DEX (25, 37.5, 50, 75, 100 µg mL^{-1}).

The calibration curves show a linear correlation of the analytical signal for all compounds. The LOD was calculated according to the IUPAC criteria as 3.0 times se/b1 and the LOQ was determined as 10 times se/b1 where se is the square root of the residual variance of the standard curve and b1 is the analytical sensitivity. The LOD values achieved by the proposed methodology is sufficient for the determination of compounds (Muñoz *et al*., 2019). The precision of the method, which includes the intra and inter day

repeatability, is expressed as the RSD of DEX, EPH, CHL, PSE, 4-AP, PAR determinations in the standard samples in accordance with the procedure described in the validation studies section. Regression parameters of the calibration curves are displayed in Table II.

Table II - Results of regression parameters

Regression parameters	PAR	4-AP	PSE	CHL	EPH	DEX
Regression equation	y=4.22x +3.45	y=6.94x - 0.73	y=8.58x - 8.64	y=15.03x - 3.93	y=6.01x - 0.23	y=3.66x +7.21
Determination coefficient (R^2)	0.9996	0.999	0.9997	0.9995	0.9996	0.9991
Calibration range (µg mL^{-1})	50-900	5-20	25-120	2-10	5-20	25-100
LOD (µg mL^{-1})	12.40	1.00	5.10	0.40	1.10	4.50
LOQ (µg mL^{-1})	37.50	2.90	15.60	1.20	3.40	13.60
Repeatability intra-day (RSD% n=3)	0.04	0.47	0.08	1.39	0.7	0.16
Repeatability inter-day (RSD% n=3)	0.17	0.63	0.11	1.31	1.01	0.49

Analysis of pharmaceutical dosage forms

In order to evaluate the applicability, recovery and possible matrix effect of the proposed CE method, the commercial tablet (Gripamol®) with reported amount of 325 mg PAR, 30 mg PSE, 2 mg CHL and 15 mg DEX was examined for assay analysis. Obtained electropherograms were presented in Figure 3. The quantifications (Table III) for each analyte were calculated from the calibration curves.

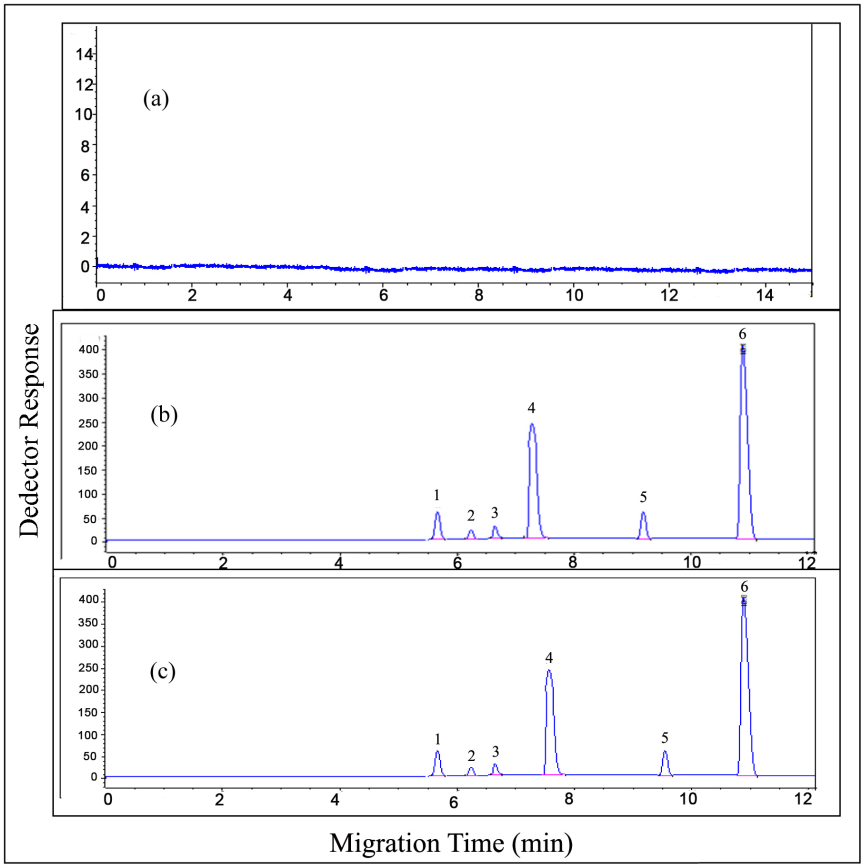

Figure 3 - Electropherograms at λ=192nm, a)BGE solution, b)Standard solutions of DEX, EPH, CHL, PSE, 4-AP, PAR, respectively, c)Solution containing pharmaceutical preparation (Gripamol tablet) and impurities, Conditions: t_{inj}=10 s, Hydrodynamic injection P_{inj}=50 mbar, Isolation Voltage=30 kV, Capillary diameter: 75 µm, BGE: 75 mM NaBorat (pH: 8.0) buffer-15% ACN, T=30ºC

Table III - Results of quantifications studies and recovery studies

Parameters	Quantifications results						Recovery results					
	PAR	4-AP	PSE	CHL	EPH	DEX	PAR	4-AP	PSE	CHL	EPH	DEX
Claimed amount (mg tablet^{-1})	325.00	6.00	30.00	2.00	6.00	15.00	100.00	100.00	100.00	100.00	100.00	100.00
Found amount* (mg tablet^{-1})	325.16	5.99	29.77	2.02	5.99	14.82	99.92	99.76	99.62	100.57	100.10	100.14
Bias (%)	0.83	0.019	0.74	0.13	0.05	0.82	1.38	1.09	1.49	1.40	1.15	1.91
RDS* (%)	0.09	0.31	0.09	0.09	0.83	0.09	1.49	1.09	1.49	1.39	1.14	1.90

* Each value is the mean of seven experiments

In order to determine the accuracy of the developed method, recovery studies were conducted. For this purpose, known amounts of standard PAR, 4-AP, PSE, CHL, EPH and DEX solutions were added to the previously determined mixture of tablet and impurity solutions. The recovery values were calculated by using the calibration lines prepared with standard solutions from the electropherograms obtained from the injection of these solutions. The results of the recovery study are shown in Table III. As it can be seen, the recovery values obtained for each compounds are close to 100%, indicating the accuracy of the developed method.

A combination of PAR, PSE, DEX and CHL are used in pharmaceutical dosage forms for reducing symptoms which are associated with the common cold. There are many studies on determination of these compounds. From the reported methods, they are detected by HPLC, LC-MS/MS, CE or CZE. In this study, a sample preparation procedure such as liquid-liquid extraction and solid phase extraction purification is not required. This article compares the established method with the reported methods, as shown in Table IV. From Table IV, it can be seen that the sensitivity of the proposed method is high and the recoveries is satisfactory. Proposed method can meet the needs of routine analysis. The developed method offers a wider working range especially for PAR. LOQ results show that the developed method is relatively sensitive compared to other methods (Palabiyik, Onur, 2010).

Table IV - Comparison of proposed method with other methods for the determination of PAR, PSE, DEX, CHL, 4-AP and EPH

Analyte	Method	Calibration range (µg mL^{-1})	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	Retention Time or Migration Time (min)	Recovery (%)	Application	Reference
PAR	LC	4–240.00	0.10	0.29	4.19	99.80	Sugar coated tablet formulation	(Palabiyik, Onur, 2007)
DEX		0.40–19.00	0.13	0.38	7.04	99.90		
CHL		0.48–44.00	0.08	0.24	6.39	99.10		
PAR	CE	50.00-600.00	12.34	50.00	5.13	99.90-101.62	Sugar coated tablet formulation	(Palabiyik, Onur, 2010)
DEX		4.00-20.00	1.94	4.00	3.31	100.21-102.10		
CHL		1.60-8.00	0.007	1.60	3.51	99.55-101.62		
PSE	CE	8.00–520.00	1.94	-	~ 3.90	92.00-98.00	Tablet formulations	(Dong *et al*., 2005)
DEX		2.50-160.00	0.98	-	~ 5.00	91.00-109.00		
CHL		4.50-300.00	1.19	-	~ 6.50	91.00-105.00		
PAR	Normal-phase LC	250.00-750.00	-	-	~ 1.50	99.20	Tablet formulation	(Al-Rimawi, 2010)
PSE		150.00-450.00	-	-	~ 3.50	99.70		
CHL		10.00-30.00	-	-	~ 7.00	98.10		
DEX		75.00-225.00	-	-	~ 5.50	98.60		
PAR	CE	50.00-900.00	12.40	37.50	5.83	99.92	Tablet formulation	This study
4-AP		5.00-20.00	1.00	2.90	9.23	99.76		
PSE		25.00-120.00	5.10	15.60	7.21	99.62		
CHL		2.00-10.00	0.40	1.20	6.53	100.57		
EPH		5.00-20.00	1.10	3.40	6.05	100.10		
DEX		25.00-100.00	4.50	13.60	10.79	100.14		

CONCLUSION

The simultaneous analysis of active substances and excipients in the pharmaceutical preparation is important in routine laboratory experiments. In this study, CE method was optimized and validated for the simultaneous determination of PAR, PSE, DEX, CHL, 4-AP and EPH in pharmaceutical dosage forms. Experimental conditions were optimized by employing a step by step approach. Moreover, this method was fully validated according to the ICH guidelines under optimized conditions. Proposed method was presented numerous advantages, such as use of minimum amounts of organic solvents, rapidity, low cost, simplicity, ease of operation and high selectivity. Good recoveries, high reproducibility and interference-free electropherograms were also achieved. High percentage of recovery results showed that the proposed method was free from interferences of commonly used excipients and additives in the formulations. Finally, new CE method was developed for the first time for analysis of selected compounds. The proposed method is suitable for quality control laboratories where economy and time are essential.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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