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Evaluation of Chemotherapy Drugs Carboplatin and Temozolamide in Pharmaceutical Formulations and Biosamples via Capillary Zone Electrophoresis Approach

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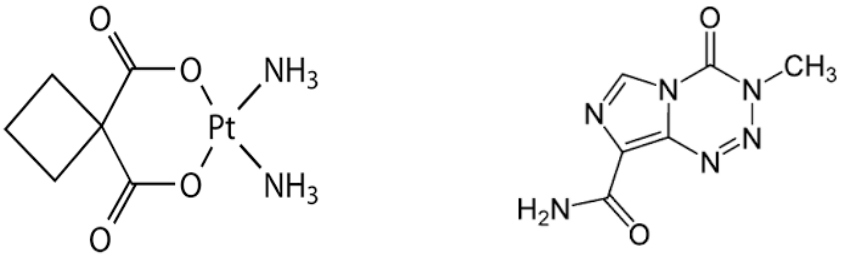
A simultaneous separation process of two anticancer drugs carboplatin and temozolamide in their binary mixture using capillary zone electrophoresis method has been introduced in this study. Under full optimized conditions a fused silica capillary was used to carry out the electrophoretic separation using a background electrolyte 50 mmol L^{-1} acetate buffer of pH = 3.6 which was run at potential 20 kV, hydrodynamic injection 8 s and 60 mbar pressure. The capillary cartridge temperature was adjusted at 30˚C and a diode array detector was utilized to record the separation peaks in the presence of internal standard phenylethylamine solution at 330 nm. Excellent electrophoretic separation was achieved over linear concentration ranges of 10-240 and 5-100 µg mL^{-1} with correlation coefficients of 0.9996 and 0.9993 for carboplatin and temozolamide, respectively. The obtained detection and quantification limits were found to be 2.5 and 7.4 µg mL^{-1} for carboplatin, while, for temozolamide were found to be 1.3 and 4.3 µg mL^{-1}. This analytical approach was validated and the results were compared with those reported by other reported methods.

Keywords: Capillary zone electrophoresis; Carboplatin; Temozolamide; Chemotherapy; Pharmaceutical formulations; Biological fluids

1. INTRODUCTION

Malignant gliomas are considered as the most predominant and fatal type of brain tumor in the adults [1]. To treat various types of cancers, including brain tumors, the DNA alkylating agents are very important to inhibit the genetic formation of the cells [2, 3]. Recently, the combination of both carboplatin and temozolamide are considered the most promising alkylating agents for the treatment of brain tumors [4].

Carboplatin (CRP) as in (Figure 1a) acts as a chemotherapy alkylating agent which is recommended for various types of cancer tumors [5, 6]. The mechanism of CRP action is explained by its activation and formation of platinum complexes inside the cells that causes intra and inter-cross linkage of DNA molecules and inhibits DNA synthesis [7].



(a) Carboplatin (b) Temozolamide

Figure 1. Chemical structures of carboplatin and temozolamide

The literature survey addressed some chromatographic techniques, for the detection of CRP in different matrices. Among these techniques is HPLC detection of CRP in an amphiphilic polymer matrix [8], canine plasma [9] and injections [10]. Furthermore, LC-tandem mass spectrometry was reported for the detection of CRP in plasma and tumor [11, 12]. On the other hand, few spectroscopic methods were reported for the detection of CRP such as atomic absorption spectrometry [13] and spectrophotometry [14].

Temozolamide (TMZ) as illustrated in (Figure 1b) is a drug which slowing and stopping the cancer cell growth in the body by inhibiting the formation of new DNA in the cells. [15]. Many developed methods for the detection of TMZ were published, including chromatographic methods such as HPLC [16-18] and UPLC-MS/MS [19]. Moreover, few spectroscopic articles were reported such as spectrophotometry [20, 21]. The developed capillary zone electrophoresis (CZE) method has many advantages, such as minimizing solvent consuming, cost benefit technique and high separation speed. The literature survey revealed that no CZE method has been published yet for the detection of both CRP and TMZ. Therefore, in this study, our point of view is the suggestion of a novel, simple, sensitive and reproducible method for the simultaneous separation and assay of CRP and TMZ. The suggested method was encouraged to be ensured with respect to ICH guidelines [22].

2. EXPERIMENTAL

2.1. Instrumentation

The developed CZE method was applied for the detection of CRP and TMZ using PrinCE 770-Technology instruments which connected with diode-array (DAD) detector. The temperature was adjusted by thermostated column cartridge and the system was automatically controlled. Data acquisition was performed using the device (WinPrinCE-770, DA×3D) software. The pH adjustment

throughout the experiment was conducted using HANNA microprocessor pH-meter model 211 (Cluj, Romania).

2.2. Chemicals and Reagents

In the detection of CRP and TMZ, all solvents are HPLC spectroscopic grade and analytical grade chemicals were used. Deionizied water was used through this study. Acetate buffer pH = 3.6 was applied as an electrophoretic background solution and prepared using 0.1 mol L^{-1} of glacial acetic acid as well as sodium acetate. Other buffers were tested, including phosphate buffer pH = 5.8 and prepared by mono and dibasic hydrogen phosphate solutions. Sigma-Aldrich, Hamburg, (Germany) provided the phenylethylamine (PEA) ≥99.0%, which was used as internal standard (IS) and (BDH, Philadelphia, USA) supplied different solvents such as ethanol (EtOH), methanol (MeOH), acetonitrile (ACN) and isopropanol (IPA). Zinc sulfate ≥ 99.0%, Sodium dihydrogen phosphate, sodium hydroxide ≥ 99.0%, boric acid and sodium acetate were acquired from (WinLab, East Midlands, UK). Pure grade of CRP and TMZ were kindly provided by Tokyo Chemical Industry Co., (Tokyo, Japan). Carboplatin® injection 450 mg/45 mL and Temodar® 250 mg /capsule were purchased from local drug stores. The study of biological samples was approved by the Medical Ethics Committee of King Saud University. The informed consent was taken from all healthy volunteers before starting the study. The commercial source of the serum samples is (Randox Laboratories, Crumlin, Antrim, UK).

2.3. Preparation of All analytical samples

Standard solutions of 250 µg mL^{-1} of each bulk drug CRP and TMZ were freshly prepared by dissolving about 25 mg of each CRP and TMZ in 100 mL deionized water. The IS (PEA) of 100 µg mL^{-1} concentration 100 µg mL^{-1} was obtained by dissolving 10 mg of PEA in 100 mL deionized water. Serial dilutions were carried out to prepare the working solutions of each drug in the ranges of 10-240 and 5-100 µg mL^{-1} for CRP and TMZ, respectively using deionized water.

A standard solution of CRP injection equivalent to 250 µg mL^{-1} CRP was diluted with deionized 100 mL deionized water. The content of two Temodar® capsules was mixed well and a quantity relative to 100 µg mL^{-1} of TMZ was dissolved in deionized water and filtered. The working solutions of each investigated drug were obtained by serial dilutions in the range of 10-240 and 5-100 µg mL^{-1}, for CRP and TMZ, respectively, using deionized water. The detection using CZE in the presence of 2.0 mL of 100 µg mL^{-1} IS was performed.

Spiked serum and urine samples containing the selected drugs CRP and TMZ were subjected to analysis using the proposed CZE method. The human serum should be deproteinated prior to the analysis process by adding approximately, 1.0 mL of acetonitrile followed by 0.1 mL of sodium hydroxide (0.1 mol L^{-1}) and 1.0 mL of zinc sulfate (5.0% w/v) to one mL of serum. The solution was agitated well and centrifuged for 30 min at 3500 rpm, and then a 0.5 Milli-pore membrane filter was used to filter the clear layer. About five mL of urine sample was directly spiked separately with an accurate amount of CRP or TMZ then completed to volume with deionized water.

Both drugs were subjected to analysis using the suggested CZE method and evaluated in their binary mixture. The analysis was carried out using 250 μg mL^{-1} standard solutions of each drug in the presence of 2.0 mL of 100 μg mL^{-1} IS. CRP and TMZ standard solutions in the final concentration ratios of 1:1, 1:2, 1:3, 2:3, 2:5 and 3:4 (w/w) were mixed and the electrophoretic assay was performed.

2.4. General CZE procedure

CRP and TMZ were separated and detected in their binary mixture by the suggested CZE technique. Before the investigation of the selected drugs the capillary should be conditioned by rinsing with 0.1 mol L^{-1} sodium hydroxide and deionized water for 5 min and 2 min, respectively, and then equilibrated with running electrolyte for 5 min. Under optimum electrophoretic conditions the selected drugs were separated using background electrolyte 50 mmol L^{-1} acetate buffer of pH = 3.6. The applied potential was 20 kV and the drugs were injected hydrodynamically every 8 s under 60 mbar pressure. The electrophoretic separation of the investigated drugs was detected using a diode array detector (DAD) at 330 nm. The adjusted capillary cartridge temperature was 30 ˚C. To ensure the reproducibility of the separation process 0.1 mol L^{-1} sodium hydroxide for 5 min, deionized water for 5 min and running buffer electrolyte for 10 min were used to replenish the capillary.

2.5. Construction of calibration graphs

To construct the calibration graphs, 10-240 and 5-100 μg mL^{-1} of CRP and TMZ, respectively, were three times tested in the presence of 2.0 mL of IS. The calibration graph of each investigated drug was plotted using the peak area ratio of each concentration with respect to IS *vs.* the relating drug concentration. Furthermore, the regression equation of each graph was calculated.

3. RESULTS AND DISCUSSION

3.1. CZE separation of CRP and TMZ

The introduced CZE method was used in the presence of PEA as IS for the separation and estimation of a mixture of CRP and TMZ (60:100 μg mL^{-1}), respectively. The typical CEZ peaks of the laboratory mixture of both drugs were shown in Figure 2. Under optimum conditions excellent separation was recorded at 7.5 and 8.2 min for CRP and TMZ, respectively. The suggested technique was also used to detect CRP and TMZ in their dosage formula and bio-samples.

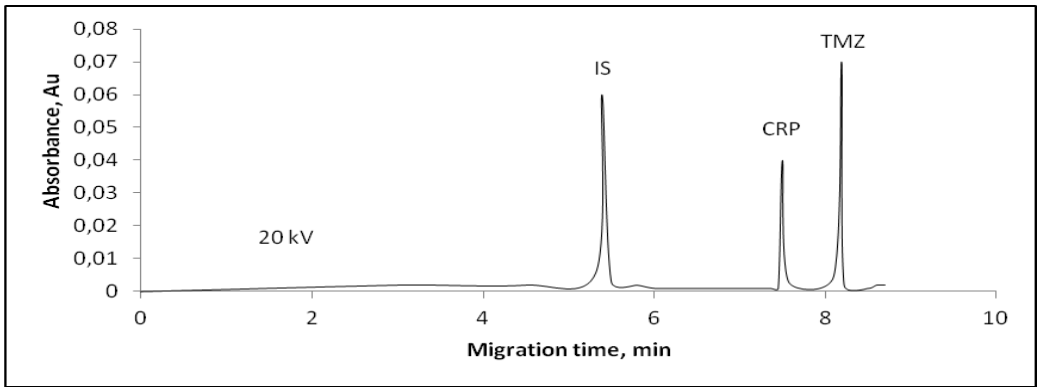


Figure 2. The obtained CZE peaks of a mixture of CRP (60 µg mL^{-1}), TMZ (100 µg mL^{-1}) and 100 µg mL^{-1} IS

3.2. Optimization of separation conditions

All method parameters including the ionization degree, electrophoretic mobility, migration time and the pH of running the buffer solution were optimized. The investigated drugs CRP and TMZ have alkaline properties. Therefore, acidic buffer can be used to separate them using CZE technique.

To ensure excellent electrophoretic separation, the suitable running buffer should be selected. Therefore, 5-70 mmol L^{-1} of acetate, phosphate and borate buffer solutions were tested by applying optimized conditions such as applied voltage, applied pressure, injection time, temperature and wavelength, etc. Reasonable results were recorded showing excellent resolution, sharp signal intensity and suitable migration time by employing acetate buffer of pH = 3.6 and 50 mmol L^{-1} concentration.

The best of our knowledge, the separation in CZE is very sensitive to pH changes rather than in other chromatographic separation such as HPLC. Therefore, the separation process can be greatly affected by a small change in pH. Also, the pH value of the running buffer was examined to ensure excellent separation of the selected drugs. The mobility (u_{eff}) curve of CRP, TMZ and IS was plotted indicating good separation by using an acetate buffer of pH = 3.6 (Figure 3).

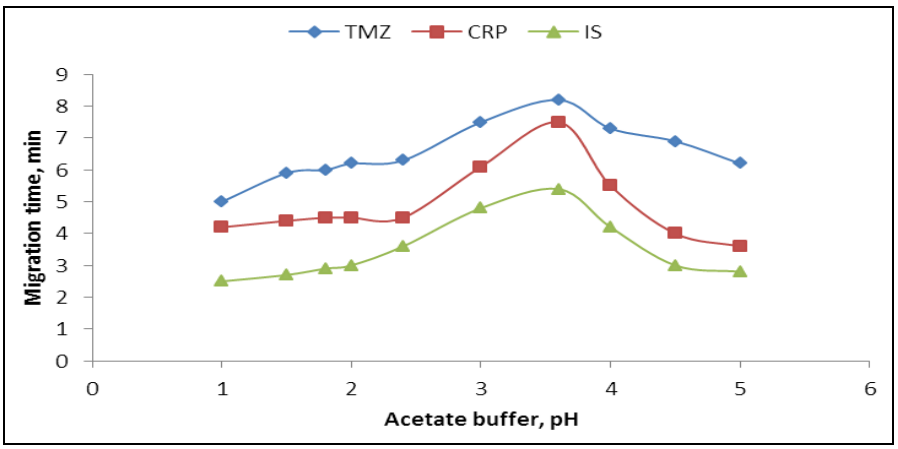


Figure 3. The influence of buffer pH on the migration time using the optimum experimental conditions

The concentration of the running buffer is greatly influenced the separation process in CZE. The mechanism of its action is controlled by a stacking phenomenon which is explained by keeping the conductivity of the buffer more than the conductivity of the sample. On the other hand, EOF is a factor which related to the concentration of buffer and also influences the separation process as well. The separation of the drugs was increased by increasing buffer concentration but at the same time decrease the EOF within the capillary was observed. To select the suitable running buffer concentration, 5-70 mmol L^{-1} of acetate buffer were investigated. As shown in Figure 4, high separation with reasonable migration time was obtained under constant electrophoretic conditions (pH = 3.6, 60 mbar, 20 kV and 30°C) by using 50 mmol L^{-1} acetate buffer.

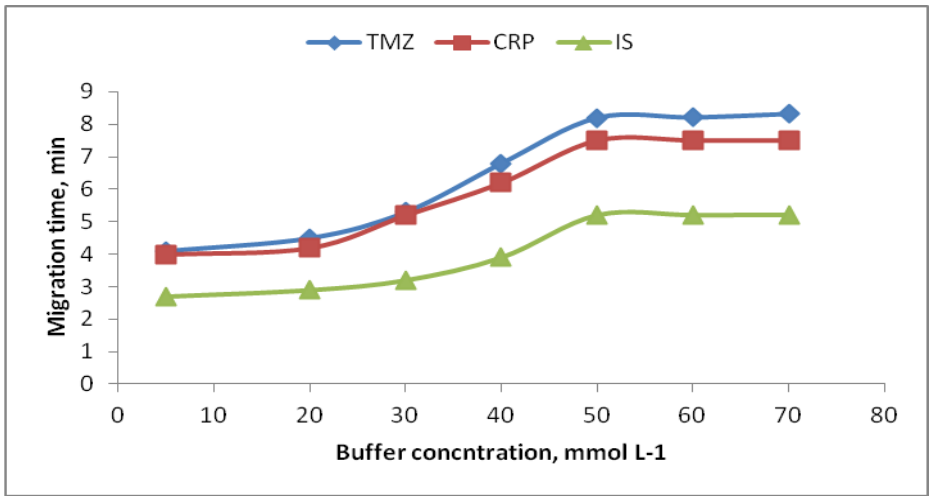


Figure 4. The influence of buffer concentration at the migration time using the optimum experimental conditions

To investigate the influence of some additives on electrophoretic system, 5-30 mmol L^{-1} sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and beta- cyclodextrin (β-CD) were tested. It was observed that no significant improvement in system separation was obtained by adding (β-CD), while, the addition of SDS and CTAB in the level above the critical micelle concentration enhanced the surfactant molecule aggregation and the interaction of the hydrophobic molecules causing a change in samples mobility [23]. Therefore, the electrophoretic separation was performed in the absence of β-CD, SDS and CTAB. Also, the addition of some organic modifiers such as ethanol (EtOH), methanol (MeOH), isopropanol (IPA) and acetonitrile (ACN) should be investigated. The movement of the interested analytes across the capillary was greatly affected by electrophoretic and electroosmotic forces. The following equation was applied to calculate the velocity of the solute:

$$V_{nett} = V_{ef} + V_{eo} = [D\zeta_{ef}/4\pi\eta + D\zeta_{eo}/4\pi\eta] E$$

Where, V_{nett} is the net movement velocity, the electrophoretic velocity is (V_{ef}) and electroosmotic velocity is (V_{eo}), η is the medium viscosity, ζ is the Zeta potential and E is the strength of the electric field.

The zeta potential and dielectric constant were decreased by adding the organic modifiers [24]. Therefore, in order to carry out this study, some organic modifiers were added to the running buffer,

with different % (10-70 v/v) of each (EtOH, MeOH, IPA and ACN). It was observed that a considerable increase in migration time and viscosity of the running buffer was obtained, but no significant improvement in the separation of both drugs was recorded by using the organic modifiers (Figure 5). Therefore, the separation process were employed without any addition of these modifiers.

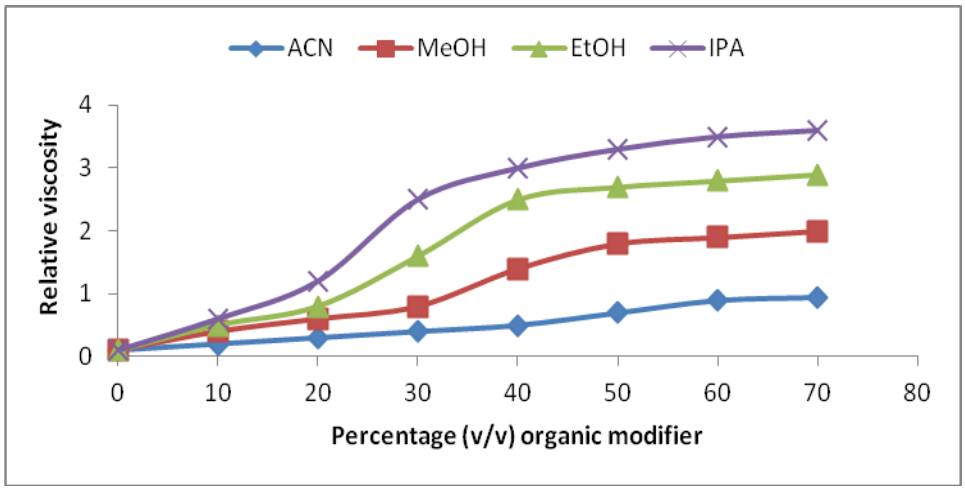
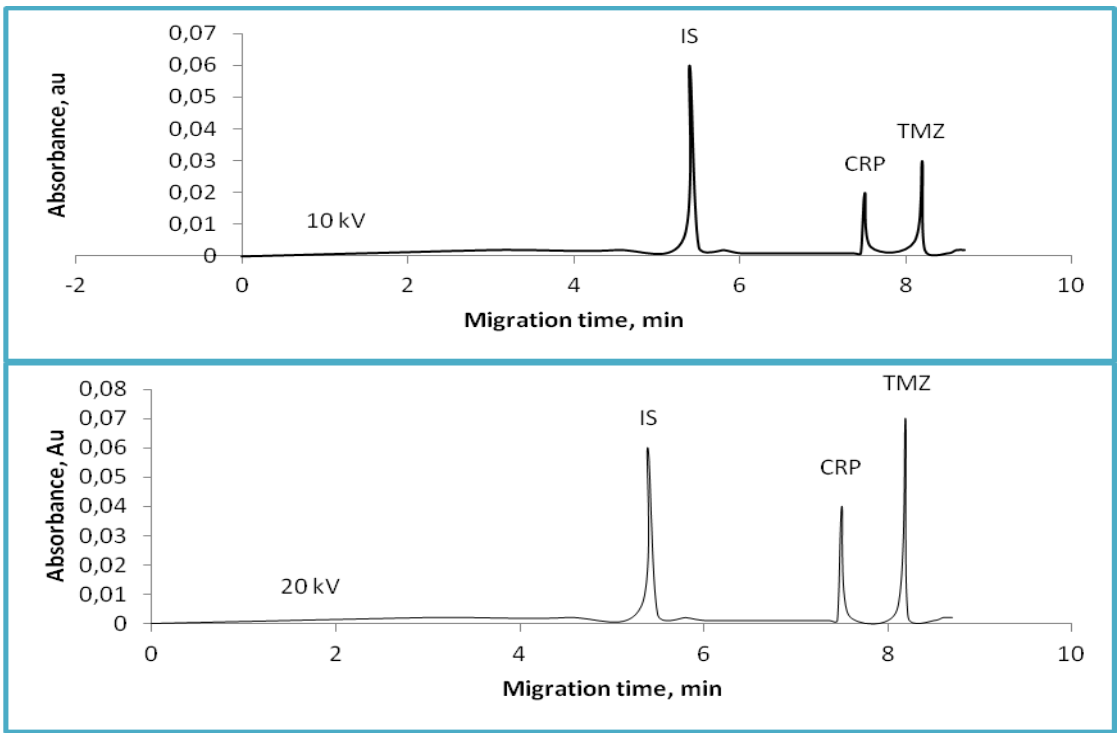


Figure 5. The influence of organic modifiers % v/v on the selected running buffer solution

The effect of applied voltage was investigated under optimum conditions by carrying out several runs using applied voltage from 10-40 kV. As previously mentioned in reference [25], a direct relationship was obtained between the applied voltage and the efficiency of resolution (Rs). Therefore, increasing the applied voltage from 10-20 kV causes an increase in the Rs.



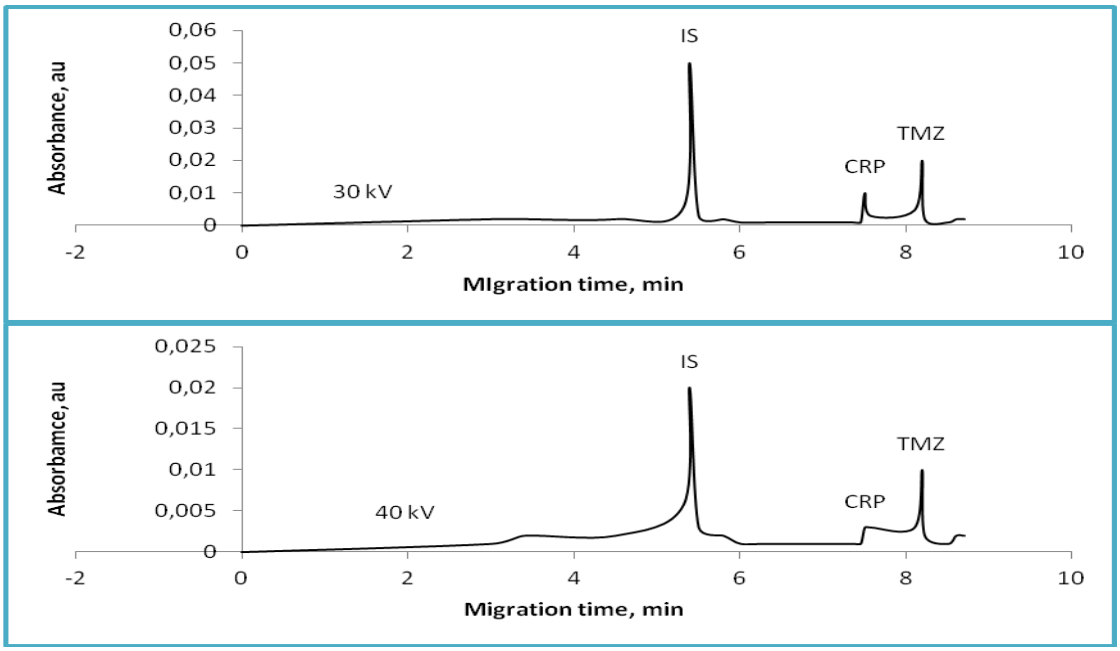


Figure 6. The selection of suitable voltage using on separation of CRP (60 µg mL^{-1}) and TMZ (100 µg mL^{-1}) and 100 µg mL^{-1} IS the optimum experimental conditions

While, excessive increasing of applied voltage than 20 kV may cause the generation of excessive Joule heat, which will cause a significant inhibition in Rs of the capillary. Figure 6 showed that 20 kV was selected as optimum voltage in this study.

The capillary cartridge temperature greatly influences the EOF and the electrophoretic mobility therefore, it should be controlled and suitable temperature should be selected. The analysis of the selected drugs CRP and TMZ using CZE was carried out using a temperature range of 25-35 ºC. Excellent separation with good resolution and reasonable migration time was achieved at 30°C.

The injection time is greatly affected the peak width and peak height in the CZE analysis. Therefore, hydrodynamic injection of the tested samples was performed in time range of 2-10 s under pressure 60 mbar. Good separation was observed after 8 s. Therefore, 8 s was chosen for further studies.

The important mission of selecting the most suitable IS is the improvement of the performance of the electrophoretic detection and separation, set off the injection error and lowers the migration time of electrophoretic separation. The pka value of PEA is 9.78 and the molar mass is less than the tested drugs, therefore, it will be expected that by using acidic electrolyte such as acetate buffer pH 3.6 the IS will be charged positively and eluted before the investigated drugs.

The suitable wavelength was selected for electrophoretic separation of the tested drugs, by carrying out the analysis at 200-400 nm. Good results and best signal to noise ratio were observed at 330 nm.

3.3. The validation of the suggested CZE Method

The introduced CZE method was validated to ensure its suitability and reproducibility according to ICH guidelines [22]. Various parameters were tested such as linearity, system stability, specificity, accuracy, and precision etc.

The linear relationship of the proposed CZE method for detection of CRP and TMZ was established by plotting the peak area ratio (drug/IS) vs. drug concentration. The applied concentration ranges were 10-240 and 5-100 µg mL^{-1} for CRP and TMZ, respectively. As demonstrated in Figure 7, excellent separation was achieved with regression equations y_{CRP} = 0.0011x + 0.3898 and y_{TMZ} = 0.0022x + 0.4364, correlation coefficients (r) 0.9996 and 0.9993 for CRP and TMZ, respectively.

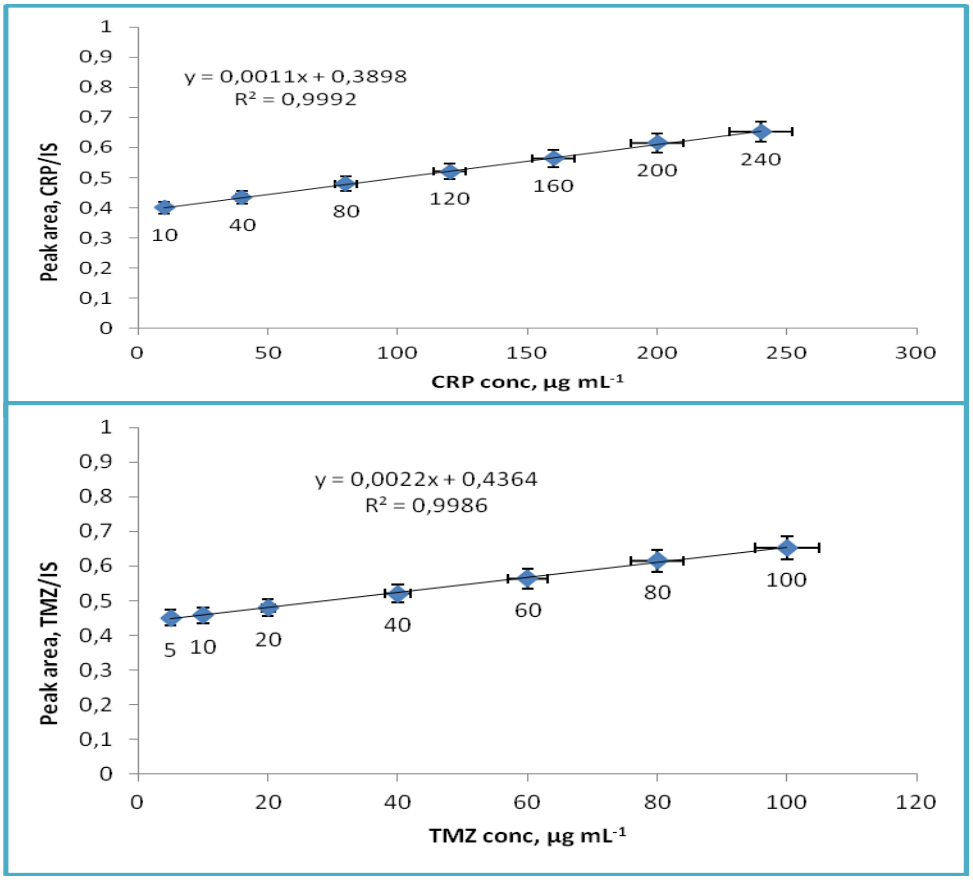


Figure 7. The plotted calibration curves of the tested drugs under optimal experimental conditions

The detection (LOD) and quantification (LOQ) limits were evaluated with respect to the guidelines ICH Q2 (R1). The resulted LOD was 2.5 and 1.3 µg mL^{-1} and LOQ was 7.4 and 4.3 µg mL^{-1} for CRP and TMZ, respectively (Table 1).

Standard solutions of the tested drugs CRP and TMZ were applied to investigate the accuracy of the suggested CZE method. The mean percentage recoveries (n = 6) were calculated and the obtained data were treated statistically and evaluated with respect to the results of other published methods, high-performance liquid chromatographic determination of CRP using Inertsil amino, 250

mm × 4.6 mm i.d. 5 µ column and mobile phase (acetonitrile : water), (85:15 v/v), flow rate 2.0 mL/min and UV-detection at 230 nm [10] and high performance liquid chromatographic determination of TMZ using C18 column, mobile phase acetic acid: methanol (50:50 v/v), flow rate 1 mL/ min and UV detection at 330 nm [16].

Table 2, listed the obtained results which revealed that no significant difference was observed. Furthermore, the precision of the electrophoretic method was investigated in the terms of inter-day and intra-day assay and the % RSD, was calculated. The results presented in Table 3 indicated a good precision of the developed CZE method was concluded.

The robustness of the suggested CZE method were investigated by inducing small changes in method parameters. The induced changes were carried out over the concentration of the running buffer (50±5 mmol L^{-1}), running buffer solution pH (3.6±0.5), the temperature of capillary cartridge 30±2 ºC, injection time 8±1 s and applied voltage 20±2 kV. No significant changes in the outcome results were noticed.

Table 1. The analytical results collected from the detection of the tested drugs using CZE method

Parameter	CRP	TMZ
Linearity range (µg mL^{-1})	10-240	5-100
Regression equation	y = 0.0011x + 0.3898	y = 0.0022x + 0.4364
Correlation coefficient (r)	0.9996	0.9993
$S_{y/x}$	0.2961	0.0032
S_a	0.0314	0.0003
S_b	0.0001	0.0002
LOD	2.5	1.3
LOQ	7.4	4.3
%RSD	0.2	0.4
%Error	0.001	0.003

Table 2. The comparative data for the detection of CRP and TMZ in their bulk powder and reference methods

Sample	Present CZE technique			Reference method				
	Taken range (µg mL^{-1})	Mean±SD	%SE	Taken range (µg mL^{-1})	Mean±SD	%SE	t-test	F-test
CRP	10-240	99.76±0.2	0.08	700-1300	99.73±0.4	0.16	0.168 (2.201)*	4.00 (4.39)*
TMZ	5-100	99.42±0.7	0.49	0.1-20	99.13±0.9	0.81	0.648 (2.228)*	1.65 (5.05)*
% SE calculated using $SD/\sqrt{n}$								
*Represent the tabulated values of t-test and F-test								

Table 3. The data recorded from the precision test for the determination of CRP and TMZ using CZE method

Parameter	CRP Taken (µg mL^{-1})			TMZ Taken (µg mL^{-1})		
Inter-day	10	100	200	5	50	100
% Found	99.60	99.18	99.14	100.00	99.29	99.88

	99.00 99.74	99.77 100.0	99.86 99.99	98.89 99.64	99.44 99.12	99.61 99.52
Mean±SD % RSD % SE	99.45±0.39 0.39 0.23	99.65±0.42 0.42 0.24	99.66±0.46 00.46 0.27	99.51±0.57 0.57 0.33	99.28±0.16 0.16 0.09	99.67±0.19 0.19 0.11
Intra-day	10	100	200	5	50	100
% Found	100.01 99.12 99.24	99.14 99.99 99.99	100.0 100.0 98.18	99.80 99.53 99.95	99.98 99.79 99.21	99.15 100.00 99.36
Mean±SD % RSD % SE	99.46±0.48 0.48 0.28	99.71±0.49 0.49 0.28	99.39±1.05 1.05 0.61	99.76±0.21 0.21 0.12	99.66±0.40 0.40 0.23	99.50±0.44 0.44 0.25

Table 4. Method robustness using 120 µg mL^{-1} CRP and 60 µg mL^{-1} of TMZ in the presence of 100 µg mL^{-1} IS

Parameter	Migration time, min		Peak area ratios	
	CRP	TMZ	CRP	TMZ
Standard	7.50	8.20	0.62	0.47
Acetate buffer pH 3.1 4.1	 7.54 7.42	 8.15 8.27	 0.65 0.61	 0.48 0.47
Acetate buffer concentration, mmol L^{-1} 45 55	 5.36 5.57	 8.12 8.23	 0.60 0.62	 0.45 0.49
Injection time, s 7 9	 7.01 7.59	 8.21 8.14	 0.69 0.64	 0.46 0.47
Applied voltage, kV 18 22	 7.34 7.13	 8.50 8.30	 0.61 0.66	 0.42 0.49
Capillary cartridge temperature, ºC 28 32	 7.53 7.61	 8.42 8.14	 0.61 0.63	 0.43 0.46

To study the specificity of the selected drugs and discriminate them from other interfering species, the standards of CRP and TMZ solutions and some possible spiking interfering compounds, including lactose anhydrous, tartaric acid, stearic acid, gelatin, sodium starch glycolate and colloidal silicon dioxide were studied. A diode array was used to record the peak purity of the samples against the standard drugs by prinCE-770 DA × 3D software. At the lower limit of quantification, no separation peaks were seen indicating high specificity of the proposed CZE method for separation of CRP and TMZ.

Under optimum conditions, the tested drugs CRP and TMZ were analyzed in their laboratory binary mixture. Sample injection was triplicated and the calibration graphs were used to calculate the percentage recoveries of each drug. As presented in Table 5, the obtained results were 99.48±0.6 and 99.23±0.9 for CRP and TMZ, respectively. Student's t-test and variance F-test [26] were applied for statistical data analysis and matched with the data obtained from reference methods [10,16] for the previously mentioned drugs.

Table 5. The obtained results of the detection of CRP and TMZ in an authentic mixture using CZE method and reference methods

Taken Ratio CRP:TMZ % w/w	CRP	Reference method (700-1300 µg mL^{-1})	TMZ	Reference method (0.1-20 µg mL^{-1})
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1:1,1:2,1:3, 2:3,2:5,3:4	99.48±0.6	99.58±0.5	99.23±0.9	98.9±1.3
t-test	0.320 (2.228)*		0.515 (2.228)*	
F-test	1.44 (5.05)*		2.09 (5.05)*	

The excellent results encouraged the detection of the selected drugs in dosage forms and bio-samples. Tables 6 and 7, presented the percentage recoveries in pharmaceutical formulations as 99.66±0.4 and 98.33±1.5 for CRP and TMZ, respectively. On the other hand, in bio-samples the outcome results in human serum were 99.7±0.4 and 99.40±0.7 and in human urine were 99.05±0.6 and 98.90±0.9 for the previously mentioned drugs, respectively.

Table 6. The obtained results of the detection of CRP and TMZ in dosage forms using CZE method and reference methods

Sample	Present CZE technique			Reference method				
	Taken range (µg mL^{-1})	Mean±SD	%SE	Taken range (µg mL^{-1})	Mean±SD	%SE	t-test	F-test
Carboplatin® injection 10-180 µg mL^{-1}	10-240	99.66±0.4	0.16	700-1300	99.53±0.6	0.24	0.451 (2.201)*	2.25 (4.39)*
Temodar® Capsule 20-140 µg mL^{-1}	5-100	98.33±1.5	0.61	0.1-20	99.37±0.7	0.29	1.539 (2.228)*	4.59 (5.05)*
% SE calculated using $SD/\sqrt{n}$								
*Represent the tabulated values of t-test and F-test								

Table 7. The data obtained from the detection of CRP and TMZ in human serum and urine using CZE method

Sample	Present CZE technique					
	CRP			TMZ		
	Taken range (µg mL^{-1})	Mean±SD	%SE	Taken range (µg mL^{-1})	Mean±SD	%SE
Serum	1:1,1:2,1:3, 2:3,3:4,3:5	99.70±0.4	0.16	1:1,1:2,1:3, 2:3,3:4,3:5	99.40±0.7	0.29
Urine	1:1,1:2,1:3,2 :3, 3:4,3:5	99.05±0.6	0.24	1:1,1:2,1:3,2: 3, 3:4,3:5	98.9±0.9	0.36

Also, to evaluate the suitability of the suggested analytical method for the separation and detection of CRP and TMZ, a comparative study was carried out between the previously addressed analytical methods and the present method. The outcome results were summarized in Table 8. The

suggested CZE method revealed high sentivity and wider concentration ranges rather than the other previously reported analytical methods for the detetion of CRP and TMZ.

Table 8. Comparative study of the suggested CZE method and the previously published methods for the detetion of CRP and TMZ

CRP			TMZ		
Principle of analytical method	Linear range	Ref.	Principle	Linear range	Ref.
RP-HPLC using C-18 column and Mobile phase:acetonitrile:water Flow rate: 2.0 mL/min Detection at: 230 nm	700-1300 (μg mL^{-1})	[10]	RP-HPLC using C-18 column and using methanol:water pH less than 3	10-100 (μg mL^{-1})	[17]
LC/MS usung a Biobasic SCX column , ion exchange chromatography flow rate of 1.5 mL/min with a gradient elution.	2-2000 (ng mL^{-1})	[12]	LC/MS/MS was used and the method based on liquid-liquid extraction.	up to 30 (mg mL^{-1})	[19]
Electrochemical atomic absorption spectrometry	Up to 80 (mg mL^{-1})	[13]	spectrophotometric detection using bromothymolblue and the detectionat 430 nm	10 - 60 (μg mL^{-1})	[21]
CZE detection using acetate buffer pH 3.6 and detection at 330 nm.	10-240 (μg mL^{-1})	Present study	CZE detection using acetate buffer pH 3.6 and detection at 330 nm.	5 - 100 (μg mL^{-1})	Present study

4. CONCLUSION

This study addressed a new CZE procedure for the detection of anti-cancer drugs CRP and TMZ in their pharmaceutical formulations and bio-samples. Excellent separation in resolution and peak areas with acceptable migration time was recorded under optimized conditions. The suggested approach was encouraged for the detection CRP and TMZ in human serum and urine and the obtained data revealed high sensitivity, accuracy and precision. System suitability and reproducibility was proved by applying method validation guidelines and the results indicated high validity for detection of CRP and TMZ.

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CONFLICT OF INTEREST
No conflict of interest associated with this work

CONTRIBUTION OF AUTHORS
The present study was carried out by all authors included in the text and all contents of this article were performed by them.

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