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Simultaneous determination of paracetamol, caffeine and propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography

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Abstract

A new micellar electrokinetic capillary chromatographic method has been developed to analyze the pharmaceutical preparations containing ternary combination of paracetamol (PAR), caffeine (CAF) and propyphenazone (PRO). Best results were obtained by using 20 mM pH 9.0 borate buffer containing 30 mM sodiumdodecylsulphate as the background electrolyte. Diflunisal (DİF) was used as internal standard (IS). The separation was performed through a fused silica capillary (50 μm internal diameter, 44 cm total length, 35.5 cm effective length) at 25 °C with the application of 3 s of hydrodynamic injection at 50 mbar pressure and a potential of 29 kV. Detection wavelength was 200 nm. Under these conditions, the migration times were found to be 5.174 min for PAR, 5.513 min for CAF, 7.195 min for DİF, and 9.366 min for PRO. Linearity ranges for the method were determined as 2–200 μg mL^{-1} for PAR and CAF and 3–200 μg mL^{-1} for PRO. Limit of detections were found as 0.6 μg mL^{-1} for PAR and CAF and 0.8 μg mL^{-1} for PRO. According to the validation study, the developed method was proved to be accurate, precise, sensitive, specific, rugged and robust. Three pharmaceutical preparations, which are produced by different drug companies in Turkey, were analyzed by the developed method. One of the same preparations was also analyzed by the derivative ratio spectro zero-crossing spectrophotometric method reported in literature. No significant differences were found statistically.

Keywords: Micellar electrokinetic capillary chromatography; Ternary mixture; Validation

1. Introduction

The ternary mixture of paracetamol (PAR), caffeine (CAF) and propyphenazone (PRO) (which have synergetic effect) is used as analgesic and antipyretic, in pharmaceutical preparations. This ternary mixture is more effective than PAR, ibuprophen and aspirin alone [1].

The determination of PAR, CAF, PRO and their mixtures with different compounds by using spectrophotometry [2–6] gas chromatography [7,8], HPLC [9–11] and various capillary electrophoresis techniques [12–17] have been described.

For the simultaneous determination of these drugs in ternary mixture preparations, derivative spectrophotometry [18,19] single flow-through UV multiparameter sensor [20] and HPLC [18,21] methods have been reported. But there is no validated capillary electrophoresis method for simultaneous determination of PAR, CAF and PRO in ternary mixtures.

Capillary electrokinetic techniques such as capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) have become popular techniques for the analysis of drug mixtures [22–24]. In MEKC, addition of micelles in the buffer solution, where the electrophoretic process takes place, allows the determination of neutral and charged analytes in a single injection [25]. Since the substances under study form a heterogeneous group, with different properties (Fig. 1), it will be difficult to separate these three drugs in a single CZE run, but presumably they can be separated by MEKC because this technique, in addition to separate neutral and ionic substances, provides more selectivity for separations.

Usually, MEKC is carried out with buffers containing some surfactant; sodium dodecyl sulphate (SDS) is the most widely used. SDS molecules form negatively charged micelles that advance against the electro osmotic force (EOF). The equilibrium established by the analytes between the micelles and

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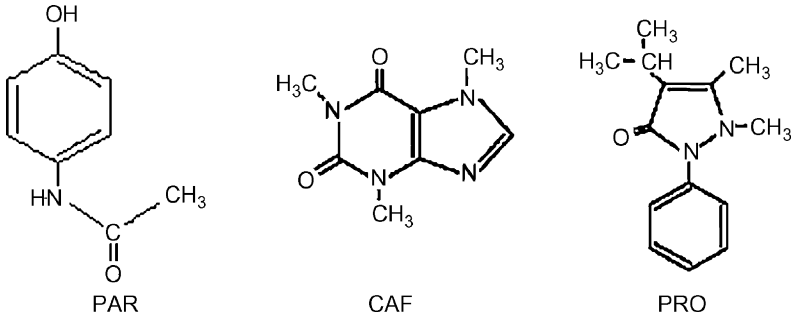

Fig. 1. Chemical structures of PAR, CAF and PRO.

the buffer, according to its hydrophobicity, determines its electrophoretic behaviour [26].

In this paper a novel, simple and rapid MEKC method has been developed for the simultaneous determination of PAR, CAF and PRO in the ternary mixture preparations using diode array detector. An optimization study of the technique variables, buffer type, pH, buffer concentration, surfactant concentration, organic modifier, injection time and applied voltage were carried out. The method was validated for specificity, linearity, sensitivity, precision, accuracy, robustness and ruggedness. The validated method was applied to the analysis of three pharmaceutical preparations including different amounts of PAR, CAF and PRO. The results of one of these pharmaceutical preparations have been compared with the derivative ratio spectro zero-crossing spectrophotometric method reported in literature [18], based on the simultaneous use of first derivative of ratio spectra and measurements of derivative ratio analytical signals corresponding to the zero-crossing points of wavelengths.

2. Experimental

2.1. Apparatus

The analyses were carried out on an Agilent Technologies ^{3D}CE (Waldbronn, Germany) using ChemStation software equipped with a diode array UV detector, an automatic sample injector Peltier temperature controller and 30 kV high voltage power supply. The fused silica capillary (44 cm × 50 μm i.d., effective length 35.5 cm) was supplied by Agilent, and the detection wavelength was set at 200 nm (bandwidth 10 nm). Sample injections were made in a hydrodynamic mode over 3 s under the pressure of 50 mbar.

Schimadzu UV-1601 UV-VIS spectrophotometry was used for the comparison method.
For pH measurements a pH meter (Mettler Toledo MA 235, Switzerland) was used.
Deionized water was prepared using a Barnstead NANOpure Diamond Analytical USA) ultrapure water system.

2.2. Chemicals

PAR, CAF and PRO were supplied by Atabay Kimya San. ve Tic. A.Ş., diflunisal (DİF), which was used as internal standard (IS), and SDS (CE grade) were purchased from Sigma. All other reagents used were of analytical reagent grade and Milli-Q water was used throughout the study.

2.3. Standard, buffer and sample preparation

2.3.1. Standard solutions

Standard stock solutions (1000 μg mL^{-1}) of PAR, CAF, PRO and DİF were prepared in water and were kept at +4 °C. Various aliquots of stock standard solutions were taken, DIF (50 μg mL^{-1}) as IS was added and then diluted to 10 mL with water to give a final desired analyte concentration.

2.3.2. Running buffer

In order to prepare 20 mM pH 9.0 borate buffer containing 30 mM SDS, 123.66 mg boric acid and 865.2 mg SDS were weighed and dissolved in 80 mL of water, the pH was adjusted to 9.0 by adding 1 M NaOH then diluted to 100 mL with water.

2.3.3. Sample preparations

Twenty tablets were weighed and grounded in a mortar, the powder equivalent to one tablet was accurately weighed and dissolved in 250 mL water with ultrosonication for 15 min. After centrifugation for 10 min at 5000 rpm, 500 μL of clear supernatant was transferred to 10 mL volumetric flask, and 50 μg mL^{-1} DIF was added and diluted to the mark with water.

Synthetic tablet solutions were prepared by mixing the solid excipients of commercial tablet form (avicel PH 101, polyvinyl pyrolydon (PVP), magnesium stearate) and solid PAR, CAF, PRO in known amounts and dissolved in water as mentioned in sample solution. All solutions were filtered through a 0.45 μm syringe filter and degassed in an ultrasonic bath for 5 min before injection to the CE system.

2.4. Operating conditions

Separations were carried out using fused silica capillary in a positive mode. The capillary was conditioned prior to its first use by flushing 1.0 M NaOH for 15 min, then with water for 20 min. Before each injection the capillary was preconditioned with 0.1 M NaOH (2 min), water (2 min) and running buffer (2 min) to maintain proper reproducibility of run-to-run injections. Injection was performed under hydrodynamic pressure at 50 mbar for 3 s. The capillary temperature was kept constant at

25 °C, and a voltage of +29 kV was applied. A diode-array UV detector was set at 200 nm with a bandwidth of 10 nm.

3. Results and discussion

3.1. Optimization of electrophoretic conditions

3.1.1. Effect of buffer pH and buffer concentration

Initially, the separation of PAR, CAF, PRO and DIF was carried out using the CZE technique. The buffer pH is one of the important parameters in CE separation, since its control determines the extent of ionization and mobility of each analytes. The influence of pH was studied using different buffers in the range of pH 2.5–10.0, and optimum separation was observed at pH 9.0 borate buffer. PAR and CAF peaks closed to each other with increasing pH, and they showed almost the same migration time at pH 10.0. In order to optimize the buffer concentration, its effect over the range 10–100 mM was investigated. When the concentration of buffer was increased, the migration times also increased. A concentration of 20 mM buffer was selected as optimal since good peak shapes, high resolution and low current were maintained. Fig. 2a shows the migration times with 20 mM pH 9.0 borate buffer. As it can be seen, PAR and CAF show similar migration times quite close to the magnitude of the EOF. It has been concluded that the separation of the four peaks by using CZE was not possible. Thus, the separation was performed by using MEKC. In this method, a buffer solution that contains micelles is used as the running buffer. The surfactant

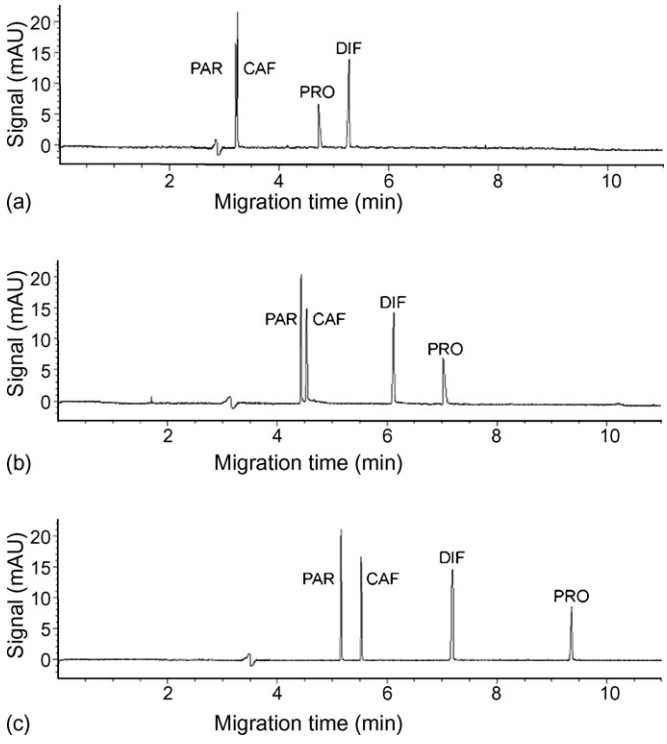

Fig. 2. Electropherograms obtained from different SDS concentrations: (a) 0 mM; (b) 20 mM; (c) 30 mM SDS. PAR, CAF, DIF, PRO concentrations 50 μg mL^{-1} each. *Conditions*: 20 mM pH 9.0 borate buffer, injection time = 3 s, pressure = 50 mbar, V = 29 kV, 25 °C, λ = 200 nm.

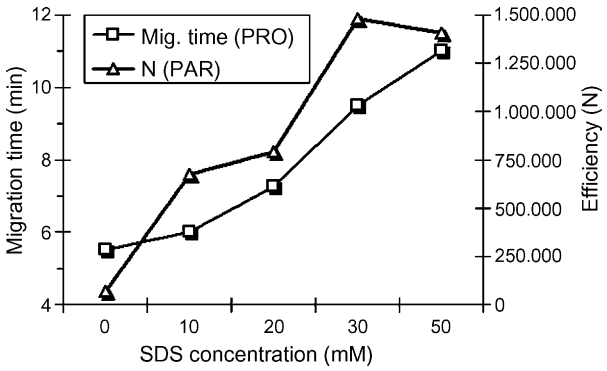

Fig. 3. Effect of SDS concentration on the analysis duration (migration time of PRO) and efficiency.

selected for this study was SDS. With this surfactant, negatively charged solutes are not strongly attracted to the micelles, and they are separated primarily as a result of differences in their electrophoretic mobilities, just as in CZE.

3.1.2. Effect of SDS concentration

The effect of SDS on the migration time and efficiency is shown in Figs. 2 and 3. In Fig. 2a, the migration times for zero SDS concentration correspond to those of the CZE method. Migration times of analytes are increased with the SDS concentration because of their solubilization by the micelles (Fig. 2b). In order to separate all the analytes in the least time possible, 30 mM of SDS (Fig. 2c) was selected as optimum since the separation with lower SDS was not enough and higher SDS increased the analysis time. In order to show the analysis time, only the migration time of the last (PRO) peak was demonstrated in Fig. 3.

3.1.3. Effect of organic modifiers

In order to investigate the effect of organic modifier, methanol and acetonitrile were added at a range of concentrations between 5–15% (v/v) to the 20 mM pH 9.0 borate buffer. In all cases, no improvements were observed, but migration times increased with the addition of organic modifiers. Therefore, no organic modifiers were added to the running buffer.

3.1.4. Effect of applied voltage and temperature

Running voltages in the range of 5–30 kV were tested by using the above conditions. As expected, increasing the applied voltage increases EOF, leading to shorter analysis time and higher efficiencies. At 29 kV, the analysis time was the shortest and the currents were not excessive (13.0 μA). So this voltage was selected as optimum running voltage.

The effect of temperature on separation was investigated at 20, 25 and 30 °C. The best resolution was observed at 25 °C.

In order to increase the sensitivity of the method, the detection wavelength was selected as 200 nm (bandwidth 10 nm) in which the analytes had maximum absorption. Through the above experiments, the optimum conditions for the simultaneous determination of PAR, CAF and PRO were decided: 20 mM pH 9.0 borate buffer containing 30 mM SDS, applied voltage 29 kV (current ca. 13.0 μA) hydrodynamic injection for 3 s at 50 mbar, working temperature 25 °C and detection

at 200 nm. Under these conditions PAR, CAF, DIF and PRO were eluted at 5.174 ± 0.006, 5.513 ± 0.004, 7.195 ± 0.007 and 9.366 ± 0.008 min, respectively (Fig. 2c).

3.2. Validation

The use of internal standard is crucial for reproducibility in CE in order to compensate the injection errors and minor fluctuations of the migration time [27]. In this study, DIF was selected as IS because of its suitable migration time. The proposed method was validated with respect to stability, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), range, precision, accuracy, recovery, robustness and ruggedness [28].

3.2.1. Stability

Stability of the standard solutions of PAR, CAF and PRO, stored at +4 °C, were evaluated at various time periods over two months. No degradation product peak has been observed during the analysis of these solutions (aged for two months) by the developed MECK method. The absorption spectra of the solutions were checked and they were found to be unchanged within this period.

3.2.2. Specificity

Specificity, described as the ability of a method to discriminate the analyte from all potential interfering substances, was evaluated by preparing the placebo, and it was confirmed that the signals measured were caused only by the analytes. A solution of analytical placebo (containing all the excipients of the formulation except the analyte) was prepared according to the sample preparation procedure and injected to the CE system. To identify the interference by these excipients, placebo (Fig. 4a), synthetic mixture (placebo, after being spiked with standards) (Fig. 4b), mixture of standard solutions (Fig. 4c) and the commercial preparations including PAR, CAF and PRO (Fig. 4d) were analyzed by the proposed method. The representative electropherograms showed no other peaks, which confirm the specificity of the method.

3.2.3. Linearity

Under the optimum analysis conditions, linearity was observed in the range of 2–200 μg mL^{-1} for PAR and CAF and 3–200 μg mL^{-1} for PRO. In all cases, 50 μg mL^{-1} DIF was added as IS. The peak normalization ratios of PAR, CAF and PRO to the DIF were plotted versus the concentration of the standards. The statistical data of the regression equations are shown in Table 1.

3.2.4. LOD and LOQ

LOD, the lowest concentration that can be distinguished from the noise level, defined as signal to noise ratio of 3:1, were 0.6 μg mL^{-1} for PAR and CAF and 0.8 μg mL^{-1} for PRO. LOQ, the lowest concentration of the substances that can be quantified with acceptable precision and accuracy, were determined with a signal-to-noise ratio 10:1, as 2 μg mL^{-1} for PAR and CAF (RSD 1.6%, $n = 7$) and 3 μg mL^{-1} for PRO (RSD 1.3%, $n = 7$).

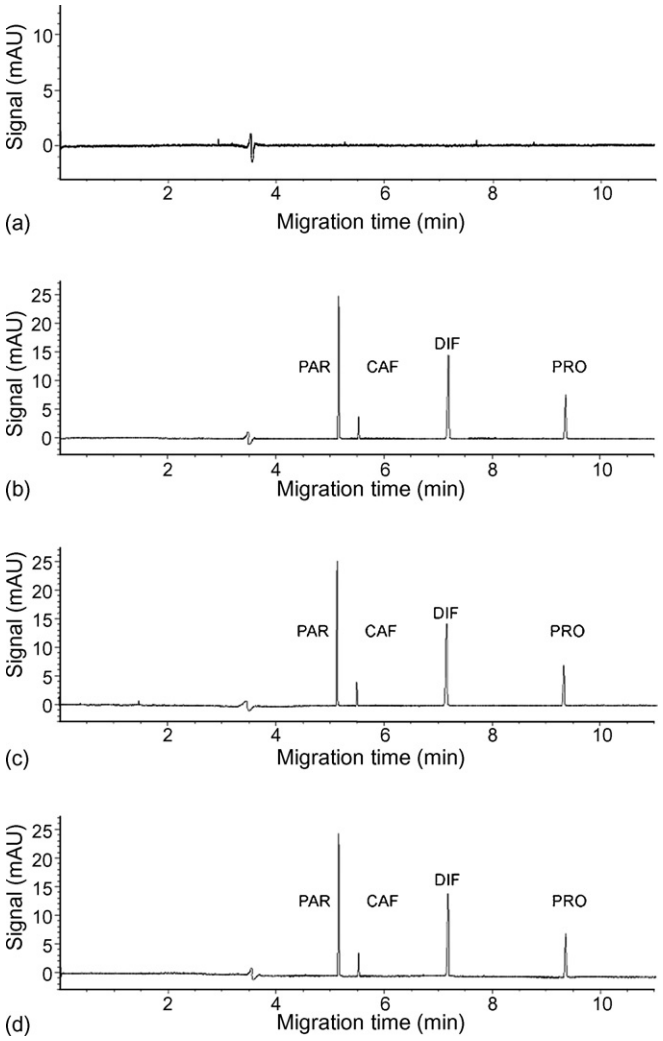

Fig. 4. Electropherograms obtained by the developed MEKC method: (a) placebo; (b) synthetic preparation; (c) standard PAR, CAF, PRO and DIF; (d) commercial pharmaceutical preparation (PAR, 50 μg mL^{-1}; CAF, 10 μg mL^{-1}; DIF, 50 μg mL^{-1}; PRO, 30 μg mL^{-1}). Running buffer; 20 mM pH 9.0 borate buffer containing 30 mM SDS; injection time = 3 s; pressure = 50 mbar; V = 29 kV; 25 °C; λ = 200 nm.

3.2.5. Precision

The assay was investigated with respect to repeatability and intermediate precision. The repeatability of the system (while keeping the operating conditions identical) was examined by ten replicate injections of the standard solution containing 50 μg mL^{-1} PAR, CAF, PRO and DIF. The results were evaluated by considering migration times (RSD < 0.6%), peak areas (RSD < 1%) and ratio of peak normalizations (RSD < 0.4%). Because of the lowest RSD of the ratio of peak normalization, this parameter was employed for quantitative procedures during the study.

Three different concentrations of PAR, CAF and PRO (in the linear range) were analyzed in six independent series in the same day (intra-day precision) and in six consecutive days (inter-day precision). Within each series every sample was injected three times. The RSD values of intra- and inter-day studies varied from 0.1 to 1.76%, showing

Table 1
The statistical data of the regression equations for PAR, CAF and PRO obtained by developed MEKC method ($n = 7$)

Substance	Regression equation[a]	Standard error of slope	Standard error of intercept	Correlation coefficient (r)
PAR	$y = 0.0209x = 0.0037$	0.0001	0.0009	0.9999
CAF	$y = 0.0181x = 0.0043$	0.0001	0.0005	0.9998
PRO	$y = 0.0127x = 0.0029$	0.0003	0.0004	0.9999

[a] y: ratio of peak normalization; x: concentration ($\mu g\,mL^{-1}$).

Table 2
Accuracy and precision data for PAR, CAF and PRO obtained by developed MEKC method ($n = 6$)

Added ($\mu g\,mL^{-1}$)	Intra-day			Inter-day		
	Found ($\mu g\,mL^{-1}$), $\bar{x} \pm$ SE	Precision[a], RSD%	Accuracy[b], bias%	Found ($\mu g\,mL^{-1}$), $\bar{x} \pm$ SE	Precision[a], RSD%	Accuracy[b], bias%
PAR						
10	10.06 ± 0.06	1.42	0.60	10.03 ± 0.07	1.71	0.30
50	50.07 ± 0.11	0.52	0.14	49.92 ± 0.30	1.48	−0.16
100	100.12 ± 0.12	0.29	0.12	99.88 ± 0.44	1.08	−0.12
CAF						
10	9.99 ± 0.03	0.83	−0.10	9.98 ± 0.15	1.76	−0.20
50	50.08 ± 0.18	0.88	0.16	50.08 ± 0.34	1.67	0.16
100	100.06 ± 0.23	0.57	0.06	100.04 ± 0.45	1.10	0.04
PRO						
10	10.03 ± 0.06	1.37	0.30	10.03 ± 0.07	1.69	0.30
50	50.55 ± 0.20	0.95	1.10	50.31 ± 0.35	1.73	0.62
100	100.08 ± 0.23	0.57	0.08	100.01 ± 0.04	0.10	0.01

[a] RSD%; relative standard deviation.
[b] Bias%; [(found − added)/added] × 100.

that intermediate precision of the method was satisfactory (Table 2).

3.2.6. Accuracy

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percent difference (bias %) between the measured mean concentrations and the corresponding nominal concentrations [29]. Table 2 shows the data obtained for intra- and inter-day accuracy.

3.2.7. Recovery

The accuracy of the method was also tested by recovery experiment. Recovery studies were performed by adding known amounts of PAR, CAF, PRO and IS to the analytical placebo solution. These synthetic mixture solutions were treated as described in the procedure of sample preparation. The results were summarized in Table 3. Closeness of the recovery results to 100% showed that recovery of the method was very good.

3.2.8. Robustness and ruggedness

Robustness is the capacity of the method to remain unaffected by small but deliberate variations introduced into the method parameters [30]. Several experimental parameters like buffer pH, buffer concentration, SDS concentration, detection wavelength, voltage and temperature were varied around the optimum value in the method to reflect changes likely to arise in different test environments. Analysis was carried out in triplicate, and only one parameter was changed at a time in the experiments. The migration times and peak normalizations relative to the IS were examined under the various conditions. The statistical comparison was done with Kruskal Wallis Varians Analysis and no differences were found between the results. ($KWT > KW$; $P < 0.05$) (Table 4). It can be said that the method developed is robust to the small changes in experimental conditions.

The analysis was performed by another analyst in order to test ruggedness of the method. The data obtained by different analysts were evaluated by Wilcoxon's Test and no differences were found (Table 3).

Table 3
Recovery and ruggedness data for PAR, CAF and PRO obtained by two different analysts ($n = 7$)

The data of first analyst				The data of second analyst			
Added (mg)	Found (mg), $\bar{x} \pm$ SE	Recovery (%)	RSD%	Found (mg), $\bar{x} \pm$ SE	Recovery (%)	RSD%	T_C
PAR 300	299.81 ± 0.08	99.94	0.15	299.82 ± 0.07	99.94	0.15	25
CAF 50	49.86 ± 0.10	99.72	0.39	49.89 ± 0.13	99.78	0.34	21
PRO 150	149.83 ± 0.12	99.89	0.17	149.81 ± 0.11	99.87	0.18	23
			$T_T = 6$; $P > 0.05$				

T_C, $T_{calculated}$; T_T, $T_{tabulated}$.

Table 4
Robustness data of the developed MEKC method (PAR, CAF, PRO and DİF 50 μg mL^{-1} each)

	PAR				CAF				PRO			
	Ratio of migration time		Ratio of peak normalization		Ratio of migration time		Ratio of peak normalization		Ratio of migration time		Ratio of peak normalization	
	$\bar{x}$	RSD%	$\bar{x}$	RSD%	$\bar{x}$	RSD%	$\bar{x}$	RSD%	$\bar{x}$	RSD%	$\bar{x}$	RSD%
Standard conditions	0.72	0.13	1.04	0.96	0.77	0.11	0.89	0.00	1.30	0.24	0.63	0.00
pH 8.9	0.72	0.10	1.04	0.85	0.77	0.21	0.89	0.12	1.29	0.20	0.62	0.08
pH 9.1	0.72	0.15	1.03	0.75	0.77	0.15	0.89	0.05	1.30	0.18	0.63	0.02
19 mM borate buffer	0.71	0.23	1.04	1.03	0.76	0.30	0.89	0.03	1.28	0.27	0.63	0.03
21 mM borate buffer	0.72	0.11	1.03	0.91	0.78	0.23	0.89	0.07	1.30	0.15	0.63	0.12
29 mM SDS	0.71	0.18	1.04	0.90	0.76	0.17	0.89	0.11	1.29	0.24	0.63	0.17
31 mM SDS	0.73	0.22	1.04	0.76	0.77	0.21	0.90	0.06	1.31	0.30	0.64	0.05
198 nm detection	0.72	0.13	1.05	0.90	0.77	0.10	0.89	0.04	1.30	0.17	0.61	0.01
202 nm detection	0.72	0.13	1.03	1.07	0.77	0.09	0.89	0.11	1.30	0.21	0.64	0.08
28 kV voltage	0.72	0.12	1.04	0.75	0.77	0.14	0.89	0.20	1.30	0.26	0.63	0.14
30 kV voltage	0.72	0.15	1.04	0.89	0.77	0.20	0.89	0.14	1.30	0.24	0.63	0.08
24 °C temperature	0.73	0.37	1.04	0.66	0.77	0.09	0.90	0.04	1.30	0.17	0.62	0.21
26 °C temperature	0.71	0.23	1.04	1.08	0.77	0.12	0.89	0.10	1.28	0.34	0.63	0.17
Kruskal Wallis varians analysis ($KW_T = 21.03$)	$KW = 8.64$		$KW = 11.15$		$KW = 6.95$		$KW = 1.15$		$KW = 15.75$		$KW = 8.71$	

KW_T, tabulated value; KW, calculated value.

Table 5
The data of analysis of commercial tablets by MEKC and Comparison method ($n = 9$)

	Labeled claim (mg)	Found					
		MECK method		Comparison method			
		mg, $\bar{x} \pm$ SE	RSD%	mg, $\bar{x} \pm$ SE	RSD%	T_H	
PAR	250	250.72 ± 0.37	0.47	250.12 ± 0.84	1.06	19	
CAF	50	49.96 ± 0.15	0.95	49.94 ± 0.16	1.00	26	$T_T = 8$
PRO	150	149.88 ± 0.28	0.59	149.63 ± 1.64	3.47	25	$P > 0.05$
PAR	300	300.12 ± 0.17	0.18				
CAF	30	30.10 ± 0.05	0.51				
PRO	150	150.14 ± 0.18	0.38				
PAR	300	299.9 ± 0.33	0.35				
CAF	50	50.20 ± 0.07	0.46				
PRO	150	149.94 ± 0.16	0.33				

3.3. Analysis of pharmaceutical preparations

The developed and validated method was applied to the simultaneous determination of PAR, CAF and PRO in three different pharmaceutical preparations, which contain these substances in different amounts. Each pharmaceutical preparation was analyzed with ten independent determinations and each series were injected three times. Satisfactory results were obtained for each compound and were found to be in agreement with label claims (Table 5). For one of the dosage forms, derivative ratio spectra zero-crossing spectrophotometric method mentioned in literature [18] was used as a comparison method to evaluate the validity of the method developed. A comparison of the results obtained by both methods was carried out using the Wilcoxon's Test. The comparison indicated no significant differences between the results obtained by the two methods (Table 5).

Table 6
The data of commercial tablet formulations obtained by calibration and standard addition techniques ($n = 3$)

Labeled claim (mg)	Calibration technique		Standard addition technique		
	MECK method, $\bar{x} \pm$ SE	Comparison method, $\bar{x} \pm$ SE	MECK method, $\bar{x} \pm$ SE	Comparison method, $\bar{x} \pm$ SE	Calculated value KW
PAR 250	250.7 ± 0.4	250.1 ± 0.8	250.9 ± 0.3	251.4 ± 0.4	0.13
CAF 50	50.0 ± 0.2	49.9 ± 0.2	50.1 ± 0.2	50.0 ± 0.2	0.15
PRO 150	149.9 ± 0.3	149.6 ± 1.6	149.9 ± 0.3	150.1 ± 0.3	0.15
			$KW_T = 3.841$; $P > 0.05$		

The results obtained from calibration technique were compared with the data obtained from standard addition technique for both developed and comparison methods. The statistical comparison of the results was done by Kruskal Wallis Varians analysis. The result showed that there were no significant differences between them (KWT > KW; $P > 0.05$) (Table 6).

4. Conclusion

A simple, fast, efficient and reliable MEKC method was developed and validated for the simultaneous determination of PAR, CAF and PRO. The method shows a good performance with respect to specificity, linearity, accuracy, precision, robustness and ruggedness. It offers a simple, fast, inexpensive and precise way for the determination of PAR, CAF and PRO in ternary mixtures. MEKC, as an alternative method to HPLC, is suitable for routine quality control and has the advantages of simplicity of operation, flexibility, low cost (requiring only a few mililiters of buffer and inexpensive capillaries) and short analysis time. Also, HPLC consumes a relatively large amount of organic solvent, which is expensive and harmful to the environment. Proposed method provides better sensitivity, accuracy, precision and a wider range of application than derivative ratio spectra zero-crossing spectrophotometric method in literature for the simultaneous determination of PAR, CAF and PRO [18].

This work also evaluates different characteristics for the validation process and outlines the specific aspects that should be considered for a MEKC methodology.

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