European Journal of Chemistry

Simultaneous determination of paracetamol, caffeine and codeine in tablets and human plasma by micellar liquid chromatography

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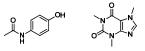
ARTICLE INFORMATION

ABSTRACT

A simple, rapid, sensitive and eco-friendly liquid chromatographic method was developed and validated for the simultaneous determination of paracetamol (PAR), caffeine (CAF) and codeine (COD). The separation was performed on cyano column using a micellar mobile phase consists of 140 mM sodium dodecyl sulfate, 25 mM phosphate buffer and 10% acetonitrile at pH = 3. The analysis was performed at a flow rate of 1 mL/min and a column temperature of 30 °C under direct UV detection at 210 nm. Total analysis time was below 6 min. Baclofen (BCF) was used as an internal standard. The validation was performed according to the ICH guidelines. The proposed method was linear over the ranges of 0.2-100.0, 0.02-12.0 and 0.2-12.0 μg/mL for PAR, CAF and COD, respectively. The limits of detection were 0.031, 0.007 and 0.054 μg/mL and limits of quantification 0.103, 0.02 and 0.164 μg/mL for PAR, CAF and COD, respectively. The results show that the procedure is suitable for the routine analysis of drugs in tablet dosage forms. The method was further extended to the determination of the studied drugs in spiked human plasma with mean percentage recoveries of 99.61±0.530, 99.28±0.523 and 99.52±0.385 for PAR, CAF and COD, respectively.

1. Introduction

Paracetamol is one of the most popular over-the-counter analgesic and antipyretic drugs (Figure 1). Paracetamol is the analgesic of choice for mild to moderate pain and for reducing fever [1]. Caffeine is a central nervous system stimulant, increasing alertness and producing agitation (Figure 1). It also relaxes smooth muscle, stimulates cardiac muscle, and appears to be useful in the treatment of some types of headache [2]. Codeine is an opioid analgesic related to morphine but with less potent analgesic properties and mild sedative effects (Figure 1). It also acts centrally to suppress cough [3]. Paracetamol-codeine-caffeine is a combination product that is used to treat mild to moderate pain, such as headache, migraine, or even surgical pain [4]. Paracetamol and codeine both act as pain killers and caffeine as a stimulant.

High Performance Liquid Chromatography (HPLC) has been highly used in the quality control of drugs because of its sensitivity, reproducibility and specificity [5]. HPLC has become an important tool for the analysis of single and various combinations of the studied drugs in pharmaceuticals or in biological fluids [6-24]. Nevertheless, only one method has been published for the simultaneous determination of the three drugs in a pharmaceutical preparation [10].

Figure 1. The structural formula of the studied drugs paracetamol, caffeine and codeine phosphate.

Micellar liquid chromatography (MLC) is one of the very important analytical techniques. Micellar media are alternative eluents to conventional aqueous-organic mixtures for the

separation of compounds of a wide range of polarities, in reversed-phase liquid chromatography (RPLC) [25-27].

Micellar mobile phases usually need less quantity of organic modifier and generate less amount of toxic waste in comparison with aqueous-organic solvents, so that they are less toxic, non-flammable, biodegradable and relatively inexpensive [28-30]. Because of these advantages, MLC is considered an interesting technique for green chemistry that copes with current concern about the environment. It proved to be a useful technique in the determination of diverse groups of compounds in several matrices [31-34].

To the best of our knowledge, no MLC method has been reported for the simultaneous determination of PAR, CAF and COD. The present study describes a rapid, simple, selective and green MLC method with UV detection for the simultaneous analysis of PAR, CAF and COD in pharmaceutical preparation, as well as in spiked human plasma samples.

2. Experimental

2.1. Instrumentation

LC analyses were performed on a Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, a pump (LC-20 AD), a column oven (CTO20AC) and UV/VIS detector (SPD-20A). This equipment has a degasser system (DGU 20 A). For data processing and acquisition, LC solution software version 1.3 from Shimadzu was used. An ultrasonicator from Merck L-7612 and a pH Meter from Hanna (USA) were used.

2.2. Materials and reagents

All the chemicals used were of analytical reagent grade, and the solvents were of HPLC grade. PAR, CAF and COD were kindly provided by El-Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt. Baclofen (BCF), used as internal standard (IS), was kindly donated by Novartis Pharma, Cairo, Egypt. Fevadol Plus tablets, manufactured by SPIMACO Al-Pharmaceutical Plant, Al- Qassime, Saudia Arabia. Triethylamine (TEA), sodium dihydrogen phosphate and orthophosphoric acid 85% were obtained from Riedel-deHäen (Sleeze, Germany). Sodium dodecyl sulphate (SDS), methanol, ethanol, *n*-propanol, butanol, tetrahydrofuran and acetonitrile (HPLC grade) were obtained from Sigma-Aldrich (Germany). Human plasma samples was kindly provided by Mansoura University Hospitals, Mansoura, Egypt and were kept frozen (-20 °C) until used after gentle thawing.

2.3. Chromatographic conditions

Chromatographic separations were achieved on a Shim-pack Cyano column (150 mm × 4.6 mm i.d.) from Shimadzu. The mobile phase consists of 25 mM sodium dihydrogen phosphate buffer 140 mM SDS, 10% acetonitrile. The pH of the mobile phase was adjusted to pH = 3.0 using orthophosphoric acid and the flow rate were 1 mL/min. The column was operated at 35 °C and the wavelength was adjusted at 210 nm.

2.4. Preparation of standard solutions

Stock standard solutions of PAR, CAF, COD and BCF (IS) (1 mg/mL) were prepared in methanol separately with the aid of ultrasonic bath. Working standard solutions for the analytical application 200 µg/mL of CAF, 200 µg/mL of COD and 500 µg/mL of PAR were prepared by appropriate dilution with the mobile phase. Standard laboratory prepared mixture solutions were prepared by mixing appropriate volumes of PAR, CAF and COD working standard solutions in 10 mL volumetric flasks and diluting to the volume with the mobile phase keeping the medicinally recommended ratios of 50.0:3.0:0.8 for PAR, CAF and COD, respectively. All solutions were stored

in the refrigerator and were found to be stable for at least 5 days without alteration.

2.5. Procedures

2.5.1. Preparation of calibration graphs

Accurately measured aliquot volumes of the suitable drug working standard solutions were transferred into a series of 10 mL volumetric flasks, so that the final concentration was in range of 0.2-100.0 µg/mL for PAR, 0.02-12.00 µg/mL for CAF and 0.2-12.0 µg/mL for COD. To each flask, 5 µg/mL (final concentration) of BCF standard solution was added as internal standard. Then, the solutions were completed to the volume with the mobile phase. Aliquot of 20 µL were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The average peak area ratio (Drug/IS) versus the final concentration of the drugs in µg/mL was plotted. Alternatively, the corresponding regression equations were derived.

2.5.2. Preparation of sample solutions

Ten Fevadol® Plus tablets were weighed then crushed to a fine powder. An accurately weighed amount of the powder equivalent to 500, 30 and 8 mg of PAR, CAF and COD, respectively which (The content of one tablet) transferred to 100 mL volumetric flask and 80 mL of methanol was added. The solutions were sonicated for 30 min and vortex mixed for 15 min, and then diluted to the mark with methanol. The solutions were filtered through Whatman filter paper. Aliquots containing suitable concentrations of the studied drugs were analyzed as described under "Construction of calibration graphs". The nominal content was calculated either from a previously plotted calibration graph or using corresponding regression equation.

2.5.3. Assay of PAR, CAF and COD in human plasma samples

The spiked plasma sample (500 µL) was diluted to 10.0 mL with the mobile phase to give a final concentration range of 0.2-2.0, 0.02-0.20 and 0.2-0.8 µg/mL for PAR, CAF and COD, respectively, with a constant concentration of BCF (0.5 µg/mL), then vortex mixed. The solutions were filtered through a 0.45 µm cellulose acetate Syringe filter. Aliquots of 20 µL were injected (in triplicate) and eluted with the mobile phase under the reported chromatographic conditions. A blank experiment was carried out simultaneously. The peak area ratios were plotted versus the concentration of the drug in µg/mL. The corresponding regression equations were derived.

3. Results and discussion

A micellar mobile phase has been utilized in this study for determination of PAR, CAF and COD together. Figure 2 shows that the three drugs were well separated under the described chromatographic conditions.
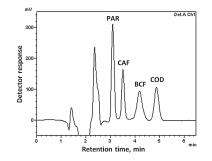

Figure 2. Typical chromatogram of standard PAR (100 µg/mL), CAF (6 µg/mL) and COD (1.6 µg/mL) using BCF (IS, 5 µg/mL).

3.1. Method development and optimization

3.1.1. Choice of column

Trials were performed using three different columns including: Shimadzu VPODS C_{18} column (150 mm × 4.6 mm i.d.), Shimadzu, Kyoto, Japan, CLC-C_8 column (150 mm × 4.6 mm i.d.), Shimadzu, Kyoto, Japan and Shim-pack cyano column (150 mm × 4.6 mm i.d.). The C_{18} column resulted in low resolution between PAR and solvent peak with long retention time for COD (16.8 min). On the other hand using C_8 column resulted in low resolution between PAR and CAF. The CN column was the most suitable one since it produced symmetrical peaks with high resolution and high sensitivity within a reasonable analysis time. As a consequence, CN column was used for further method development and optimization.

3.1.2. Mobile phase composition

Several modifications in the mobile phase composition were performed in order to study the possibilities of improving the performance of chromatographic system. These modifications included the change of the type and % concentration of organic modifier, the concentration of SDS, concentration of buffer and pH of the mobile phase.

3.1.3. Type of the organic modifier

Different organic modifiers were tried during the experimental study to choose the most suitable one for chromatographic separation of the three drugs. The studied organic modifiers included methanol, ethanol, *n*-propanol, butanol, tetrahydrofuran, and acetonitrile. It was found that using methanol or ethanol showed delay in retention time of COD. In addition, *n*-propanol, butane land tetrahydrofuran gave lower sensitivity and slight overlapping between PAR and CAF. So, acetonitrile was the organic modifier of choice giving good resolved and highly sharp peaks within a reasonable time.

3.1.4. Percentage of the organic modifier

The effect of changing the percentage of acetonitrile on the retention times and selectivity of the test solutes was investigated using mobile phases containing 5-20% of acetonitrile. It was found that the resolution between PAR and CAF is decreased upon increasing the % volume of acetonitrile. The study revealed that the optimum chromatographic performance was achieved upon using 10% acetonitrile regarding the resolution and number of theoretical plates. Volumes less than 10% resulted in less sensitive peaks and it was time consuming.

3.1.5. Concentration of SDS

The effect of changing the concentration of SDS on the selectivity and retention times of the test solutes was investigated using mobile phases containing a concentration range of 80-160 mM SDS. It was found that, the retention time of COD increased upon decreasing the concentration of SDS. The study revealed that the optimum chromatographic performance was achieved upon using 140 mM SDS regarding the resolution of PAR and CAF and number of theoretical plates. Concentration more than 140 mM of SDS results in slight decrease in the number of theoretical plates.

3.1.6. pH of the mobile phase

The effect of changing the pH of mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 3.0 to 7.0. It was found that, for COD the retention time was not dramatically changed. While, there is a gradual decrease in PAR and CAF retention with the increase in pH from 4.0 to 6.0 which is not suitable for application in plasma. PAR and CAF peaks were not completely separated at pH = 7.0. It was found that pH = 3.0 was the most appropriate one yielding well resolved peaks and highest number of theoretical plates with good resolution between PAR and CAF.

3.1.7. Ionic strength of the buffer

Besides pH, the buffer concentration is another parameter controlling the resolution between PAR and CAF and the retention of COD. The method was investigated using mobile phases containing concentrations of 15-45 mM of phosphate buffer. It was found that the resolution between PAR and CAF decreased and retention time of COD increased upon decreasing the ionic strength of the buffer. As a compromise, 25 mM phosphate buffer was chosen as the most suitable one giving good resolution with more symmetrical peaks.

3.1.8. Flow rate

The effect of flow rate on formation and separation of peaks of studied compounds was investigated over the range 0.8-1.2 mL/min. A flow rate 1 mL/min was optimal for highest theoretical plates count and good separation in reasonable time.

3.1.9. Column temperature

The effect of column temperature (from 25 to 45 °C) on formation and separation of peaks was studied. It can be concluded that highest theoretical plates count and good separation in a short retention time and high sensitivity was obtained at 35 °C.

3.1.10. The choice of the internal standard

Different internal standards, such as meloxicam, diclofenac, celecoxibe and baclofen were investigated. It was found that, diclofenac and meloxicam had high retention time that increased the retention time of the mixture more than 6 min while celecoxibe peak was close to that of COD peak that led to overlapping between them. BCF was found to be the internal standard of choice as it produced good separation with less retention time.

3.1.11. Method validation

Validation of the proposed method was performed with respect to linearity, and range, LOD, LOQ, accuracy, precision, specificity, stability and robustness according to the international conference on harmonization (ICH) Guidelines [35].

3.1.12. Linearity and range

Under the above described experimental conditions, a linear relationship was constructed by plotting the peak area ratios of PAR, CAF and COD to the internal standard. The concentration ranges were found to be 0.2-100.0 μg/mL for PAR, 0.02-12.00 μg/mL for CAF and 0.2-12.0 μg/mL for COD. Linear regression analysis of the data gave the following equations:

$$y = 0.299x + 0.0045 \ (r = 0.9999) \text{ for PAR} \tag{1}$$

$$y = 0.450x - 0.0014 \ (r = 0.9999) \text{ for CAF} \tag{2}$$

Table 1. Analytical performance data for the determination of the PAR, CAF and COD by the proposed method.

Parameters	PAR	CAF	COD
Linearity range (μg/mL)	0.2-100.0	0.02-12.00	0.2-12.0
Correlation coefficient *(r)*	0.9999	0.9999	0.9999
Intercept *(a)*	0.0045	-0.0014	0.0212
Slope *(b)*	0.2999	0.4500	0.5475
SD of residuals ($S_{y/x}$)	0.0066	0.00413	0.0176
SD of intercept (S_a)	0.0031	0.0019	0.0092
SD of slope (S_b)	0.0006	0.0003	0.0016
% RSD (SD × 100/X`)	0.560	0.617	0.803
% Error (% RSD/√n)	0.197	0.251	0.303
Limit of detection, LOD (μg/mL)	0.031	0.007	0.054
Limit of quantitation, LOQ (μg/mL)	0.103	0.020	0.164

Table 2. Assay results for the determination of PAR, CAF and COD in raw materials.

Compound	Proposed method			Comparison method [10]
	Amount taken (μg/mL)	Amount found (μg/mL)	% Found	
PAR	0.200	0.199	99.50	99.95
	1.000	0.998	99.88	99.54
	5.00	4.94	98.80	100.98
	15.00	15.09	100.60	
	50.00	49.89	99.78	
	100.00	99.65	99.65	
	x̄±SD		99.85±0.53	100.15±0.741
	Student t-test		1.43(2.31)	
	F-test		1.83(5.14)	
CAF	0.0200	0.0199	99.95	99.95
	0.200	0.199	99.50	99.71
	0.500	0.498	99.60	100.30
	3.00	3.01	100.30	
	12.00	11.91	99.20	
	x̄±SD		99.61±0.582	99.98±0.296
	Student t-test		1.38 (2.31)	
	F-test		1.57 (5.14)	
COD	0.200	0.198	99.40	99.54
	0.400	0.398	99.50	100.10
	1.200	1.198	99.80	99.97
	4.00	4.98	99.60	
	12.00	11.87	98.90	
	x̄±SD		99.51±0.571	99.87±0.293
	Student t-test		1.461 (2.31)	
	F-test		1.982 (5.14)	

The figures between parentheses are the tabulated t and F values at *p* = 0.05 [35].

$$y = 0.547x + 0.0210 \ (r = 0.9998) \text{ for COD} \quad (3)$$

where *y* is the peak area ratio, *x* is the concentration of the drug in μg/mL and *r* is the correlation coefficient.

Statistical analysis of the data gave high values of the correlation coefficients with small intercepts indicating the good linearity of calibration graphs. Statistical analysis of the data gave high values of the correlation coefficient (*r*) of regression equations [35]. Small values of standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b) indicate low scattering of the points around the calibration curves. Also, small values of percentage relative standard deviation (RSD %) and the percentage relative errors (% Er) indicate high accuracy and high precision of the proposed method (Table 1).

3.1.13. Limit of quantification and limit of detection

The limits of quantification (LOQ) were determined by establishing the lowest concentrations that could be measured according to ICH Q2 (R1) recommendations [36] below which the calibration graphs were nonlinear. The limits of detection (LOD) were determined by establishing the minimum levels at which the analytes could be reliably detected.

$$\text{LOQ} = 10 \ S_a/b \text{ and LOD} = 3.3 \ S_a/b \quad (4)$$

where S_a = Standard deviation of the intercept of the calibration curve and b = Slope of the calibration curve.

LOQ values were found to be 0.103, 0.020 and 0.164 μg/mL while LOD values were found to be 0.031, 0.007 and 0.054 μg/mL for PAR, CAF and COD, respectively as shown in (Table 1).

3.1.14. Accuracy and precision

To prove the accuracy and utility of the proposed method, the results of the assay of studied drugs were compared with those obtained using comparison method [10]. Statistical analysis of the results obtained using Student's *t*-test and variance ratio F-test [35] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2).

Intra-day precision was assessed by analyzing three concentrations and three replicates of each concentration in one day. Also, the inter-day precision was assessed by analyzing three concentrations and three replicates of each concentration over three successive days. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed method (Table 3).

3.1.15. Specificity

Specificity was checked by analyzing PAR, CAF and COD in laboratory prepared mixtures. Good resolution and absence of interference between drugs being analyzed and other materials in the pharmaceutical formulations (Figure 3)

Table 3. Precision data for the determination of PAR, CAF and COD by the proposed method.

Parameter		PAR concentration (μg/mL)			CAF concentration (μg/mL)			COD concentration (μg/mL)		
		10	20	30	2.5	5	10	3	6	9
Intraday [a]	%Found	98.54	99.32	98.92	100.12	99.99	99.29	100.03	99.67	100.11
		99.51	98.76	99.58	99.97	100.58	100.09	98.92	99.23	100.05
		100.32	99.87	100.09	98.54	99.71	99.61	99.98	98.89	99.97
	Mean	99.45	99.31	99.53	99.54	100.09	99.66	99.64	99.26	100.04
	± SD	0.891	0.555	0.586	0.782	0.444	0.402	0.626	0.391	0.070
	%RSD	0.892	0.558	0.588	0.785	0.443	0.403	0.628	0.393	0.069
	%Error	0.516	0.322	0.339	0.453	0.256	0.232	0.362	0.227	0.040
Interday [b]	%Found	99.89	99.93	100.16	99.21	99.54	98.97	99.97	98.93	99.76
		99.74	99.65	99.87	100.01	100.09	99.91	99.85	99.62	100.05
		100.09	99.12	99.81	99.96	99.88	99.57	100.09	100.03	99.87
	Mean	99.90	99.56	99.94	99.72	99.83	99.48	99.97	99.52	99.89
	± SD	0.175	0.411	0.187	0.448	0.277	0.476	0.120	0.555	0.146
	%RSD	0.175	0.412	0.187	0.449	0.277	0.478	0.120	0.557	0.146
	%Error	0.101	0.238	0.107	0.259	0.160	0.275	0.069	0.321	0.084

[a] Within the day.
[b] Three consecutive days.

Table 4. Robustness evaluation of the proposed method.

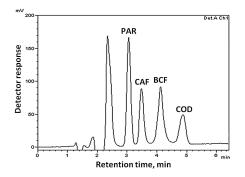| Variation	Resolution		Tailing factor			% Recovery		
	CAF/COD	PAR/CAF	PAR	CAF	COD	PAR	CAF	COD
23 mM buffer	8.4	2.4	1.08	1.09	1.03	99.61	99.80	100.15
27 mM buffer	8.2	2.3	1.03	1.04	0.99	99.00	99.11	99.88
0.145 M SDS	8.2	2.3	1.07	1.08	1.03	99.59	99.75	100.18
0.135 M SDS	8.1	2.2	1.02	1.03	0.99	99.08	99.18	99.83
11% Acetonitrile	8.8	2.9	1.03	1.05	1.01	99.41	99.76	99.98
9% Acetonitrile	8.1	2.2	0.97	1.00	0.95	99.84	99.84	100.20
pH = 2.8	8.8	2.6	1.01	1.03	0.98	99.31	99.53	100.14
pH = 3.2	8.6	2.7	0.99	1.00	0.96	99.51	99.72	100.11
Flow rate 0.8 mL	8.8	2.9	1.00	1.02	0.97	99.23	99.44	99.87
Flow rate 1.2 mL	8.5	3.1	1.01	1.03	0.99	99.32	99.51	99.98
Temperature 30 °C	8.4	2.4	1.01	1.03	0.98	99.18	99.28	99.64
Temperature 40 °C	8.9	3.0	0.99	1.01	0.96	99.50	99.70	100.11
Without variation	8.7	2.8	1.00	1.02	0.97	99.45	99.66	100.06

Figure 3. HPLC chromatogram of tablets (50 μg/mL) PAR, (3 μg/mL) CAF and (0.8 μg/mL) COD using BCF (IS, 5 μg/mL).

confirming the specificity of the method. Good recoveries were obtained in the sample indicating specificity of the proposed method. Additionally, there was not any interference encountered from human plasma matrix although no prior extraction procedure was performed.

3.1.16. Stability

Stability of the standard solutions of the studied drugs, stored at 4 °C, was evaluated at various time points over two weeks. The concentrations of freshly prepared solutions and those aged for two weeks were calculated by the method developed and the difference between them was found to be < 0.4%. These solutions can therefore be used during this interval of time without the results being affected.

3.1.17. Robustness of the method

Robustness of the method was investigated by varying the chromatographic conditions such as the flow rate, the amount

of acetonitrile in the mobile phase, the pH of the mobile phase, the temperature and the amount of SDS in the mobile phase.

In this study, the chromatographic responses monitored were resolution, tailing factor and the percent recoveries of the studied drugs. (Table 4) shows the experimental parameters performed for robustness evaluation. Therefore, little variations in the chromatographic conditions were found to be acceptable values in relation to the reference value and the developed method for analysis was considered to be robust.

3.2. Applications

3.2.1. Dosage form analysis

The proposed method was successfully applied to the simultaneous determination of PAR, CAF and COD in their co-formulated tablets as illustrated in Figure 3. The results of the proposed method were favorably compared with those obtained using the comparison method [10]. The results are abridged in Table 5. Statistical analysis of the results obtained using Student's *t*-test and variance ratio F-test [35] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 5).

3.2.2. Biological applications

Following oral administration of a single 500 mg conventional tablet or a single 650 mg extended-release PAR tablet, the average plasma concentrations was reported to be 2.1 or 1.8 μg/mL after 6 or 8 hours, respectively [37]. For CAF after oral administration of 100 mg, peak plasma concentrations of about 1.5-1.8 μg/mL are reached after 50-75 minutes [38]. While for COD, 60 mg orally, produces peak blood levels of 150 ng/mL in 30 minutes [39]. The plasma calibration curve was linear with the following equation, linear regression analysis of the data gave the following equation:

Table 5. Assay results for the determination of the PAR, CAF and COD in their co-formulated tablet by the proposed and comparison methods.

Preparation	Amount taken (µg/mL)			Amount found (µg/mL)			% Found			Comparison method [10]		
	PAR	CAF	COD	PAR	CAF	COD	PAR	CAF	COD	PAR	CAF	COD
Fevadol Plus ® tablets	100	6.0	1.6	100.13	6.010	1.589	100.13	100.16	99.35	100.01	99.94	98.89
[500 mg PAR + 30 mg CAF + 8 mg	50	3.0	0.8	50.22	2.990	0.791	100.44	99.66	98.90	100.27	99.31	99.76
COD]	25	1.5	0.4	25.02	1.502	0.398	100.08	100.13	99.74	99.93	100.15	99.81
Mean							100.21	99.98	99.33	100.07	99.80	99.48
±SD							0.195	0.280	0.420	0.177	0.437	0.517
% RSD							0.194	0.280	0.422	0.176	0.437	0.522
% Error							0.112	0.161	0.244	0.102	0.252	0.301
Student t-test							0.962	0.658	0.407			
F-test							1.204	1.748	1.515			

The values of tabulated t- and F-tests are 2.31 and 5.14 respectively at p = 0.05 [35].

Table 6. Assay Results for the determination of the studied drugs in spiked human plasma using the proposed method.

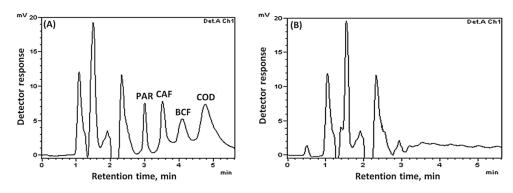| Parameter	Amount added (µg/mL)			Amount found (µg/mL)			% Found		
	PAR	CAF	COD	PAR	CAF	COD	PAR	CAF	COD
Data	0.2	0.02	0.2	0.198	0.0198	0.1991	99.01	99.45	99.55
	1.0	0.1	0.6	0.999	0.0997	0.5994	99.99	99.70	99.90
	2.0	0.2	0.8	1.997	0.1974	0.7930	99.85	98.70	99.13
Mean							99.61	99.28	99.52
±SD							0.530	0.523	0.385
% RSD							0.532	0.523	0.386
% Error							0.307	0.302	0.223

Figure 4. HPLC chromatogram of (A): PAR (2 µg/mL), CAF (0.2 µg/mL) and COD (0.2 µg/mL) using BCF (IS, 0.5 µg/mL) in spiked human plasma, (B): blank human plasma.

$$y = 0.1648x + 0.0730 \ (r = 0.9998) \text{ for PAR} \quad (5)$$

$$y = 0.5860x + 0.0803 \ (r = 0.9999) \text{ for CAF} \quad (6)$$

$$y = 0.3575x + 0.0365 \ (r = 0.9999) \text{ for COD} \quad (7)$$

where y is the peak area, x is the concentration of the drug in µg/mL and r is the correlation coefficient. The results of the analysis of PAR, COD and CAF in plasma samples are provided in (Table 6). The accuracy of the proposed method was assessed by investigating the recovery of them at three concentration levels covering the specified range. The tabulated recoveries indicate good accuracy. Figure 4 represents a typical chromatogram of three drugs and the internal standard in blank and spiked human plasma samples.

4. Conclusion

A novel and rapid micellar less hazardous and toxic liquid chromatographic method was examined for the simultaneous determination of PAR, CAF and COD. The developed method has distinct advantages regarding analysis time and cost compared with those of the previously reported methods. The method is linear over a wide range and utilizes a mobile phase which can be easily prepared. The proposed procedure, by virtue of its high sensitivity, could be applied to the analysis of PAR, CAF and COD in spiked human plasma with no need for extraction or pretreatment steps.

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