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RESEARCH ARTICLE

RP–HPLC Method Validation for Simultaneous Estimation of Paracetamol and Caffeine in Formulating Pharmaceutical Form

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ABSTRACT:
A novel, simple and accurate, Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method for simultaneous estimation of Paracetamol (PCM) and Caffeine (CAF) in mixture of standard and formulation tablets was validated in this research. The absorbance maximum of drugs using UV- spectroscopy was found at (244.8 and 272.6nm) for PCM and CAF respectively in deionized water: methanol mixture (60:40 V/V) as solvent. This method involves the separation of PCM and CAF on RP - HPLC Shimadzu type LC–20 - A, Japan, and Phenomenex column, C18 (250mm, 4.6mm and 5µm). The elution was done using an eluent phase composed of methanol and water in the ratio of (40:60 V/V with a pH adjusted at 4.0 using acetic acid). A separation was fixed for 10 min at 270nm, using a UV-Vis - detector and 1.0mL/min, flow rate and the drugs were eluted in (3.468 and 5.376 min) for PCM and CAF respectively. The suitable conditions such as the elution phase composition, rate of flow, pH and wavelength were studied. The linearity of the method was in the range of concentration within (0.5 – 25 and 0.1 - 30µg/mL), while, R^2 values within (0.9995 and 0.9997), and the means of recovery were found within (99.57 and 100.36) for PCM and CAF respectively. The method was applied for the estimation of gradient active of drugs in different formulating form samples. The method accuracy was validated by the mean of recovery percentages which, were found in acceptable limit.

KEYWORDS: Estimation, RP - HPLC, Formulating, Recovery.

1. INTRODUCTION:
It is very rare, or there is no single remedy to remediation all pain forms, as there is no typical analgesic, as each factor has the disadvantages and advantages recognize it from other analgesics to treat pain[1]. Every mechanical analgesic have its own assignment to the pain prevent. For anti-inflammatory nonsteroidal drugs, the action mechanism is in their ability to the (COX) enzyme inhibit, which in turn is primarily responsible for the prostaglandin synthesis, which is playing a key role in the group which is acting as pain converters[2]. PCM is classified as part of this group of materials[3].

PCM was used for pain joint, pain in the middle ear, head ache from analgesic effect, neuralgia, tooth ache, and aches generate from cold, tumor and flue[4]. Caffeine, which is deemed as alkaloid of Purina group[5]. CAF (1, 3, 7-trimethyxanthine), is soluble partially in water because of its moderate polarity. Caffeine is an excitant of the central system of the nerve. Since it is widely humans consumed, it is considered a most used in the world as a psychoactive substance. It was used both as a medically and as a recreationally. It was caused the vigilance increased and focusing and improved overall the coordination of body[6,7]. The combination of drugs has ultimate effective when the agents act out of different analgesic mechanization and synergistically active[8,9]. The analgesics like paracetamol is generally combined to increase the effectiveness of the analgesic[10]. Various methods of analysis are announcing to determine these drugs and other active compounds in formulating drugs like HPLC[11,12,13,14], SP-FT – Raman[15], electromagnetic[16], spectroscopy[17,18,19,20], UPLC/Q-TOF-MS[21], Electrode ion[22] and Absoption correction method[23]. The work

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objective is to evaluate a new accurate and easy chromatography analytical method for the estimation of the drug content in tablet formulated samples manufactured by different pharmaceutical corporation which available in the pharmaceutical market in Iraq, to tool up information about the different products, which may enforce or not enforce with the requirements of the formal method or other standard methods.

2. MATERIALS AND METHODS:

2.1 Chemicals and reagents:

PCM and CAF standard powder was from SDI- Iraq. Methanol (HPLC-grade) is from BDH. Sodium hydrogen phosphate, sodium borate, sodium acetate, boric acid, acetic acid, and phosphoric acid were from BDH. Deionized water, freshly prepared was used.

2.2 Instrumentation and Conditions of chromatographic:

HPLC (Shimadzu - LC – 20 - A, Japan), Germany Sartorius - balance, Karl – Kolb - Ultrasonic bath - Germany), Shaking bath water (Taiwan) and Memmert - oven – Germany, were used in this study. PCM and CAF were separated on column type Phenomenex - C-18 (250mm, 4.6mm - I.D, and 5-µm size of particle). Separation was utter at room - temperature (~25ºC) and the run time was 10 min under Reversed Phase conditions. The elution phase was methanol (MOH) and water in the ratio of (40:60 V/V) adjusted pH with acetic acid at 4.0. The rate of flow was 1.0mL/min, and an 10 µL injector loop was used for injecting samples and detection was done at 270nm. The eluent phase was degassing using the sonicator type - ultrasonic cleaner, power - sonic- 420, and then filtered over a 0.45µm filter of nylon. The identity established of the compound was done through the comparing of the standard compound solution retention time with those of a sample compound solution. Chromatography was complete in temperature column that maintained at 25±2°C. The UV- spectrums of PCM and CAF selecting the detection working wavelength were taken by the Jasco - V-650 – Japan, double - beam UV-VIS - spectrophotometer has 10 mm length path quartz cells, which was used for the analytical object.

2.3 Preparation of solutions:

Standard stock solution:

The stock standard solution has a 1000µg/mL of the PCM and CAF were prepared in the mixture of MOH and water using standard material of drugs. Transfer 10 mL of the stock solution 1000µg/mL into 100mL volumetric calibrated flask and make up to the mark with elution phase for giving a standard working solution having a100µg/mL concentration.

Diluent:

From the 100µg/mL stock solution, additional dilution was conducted through withdrawing a different volume (0.05 – 2.5 and 0.01 - 30mL) from standard solution of PCM, CAF into the series of 10mL volumetric calibrated flasks and all were complete to the mark with eluent phase to prepare standard working solutions have concentrations of (0.5 – 25 and 0.1 - 30µg/mL).

Procedure for drugs assay in pharmaceuticals tablets:

Ten tablets of PCM and CAF drug's, formula was accurately weighed and finely powdered. An accurately quantity weighed of tablets, powder which equivalent to (100mg) of PCM and CAF drugs were conveyed to a (100mL) volumetric flask and then diluted with (H_2O: MOH 60: 40 V/V), the content were ultra - sonicated for 25 min. The drugs solutions volume was completed to the mark and mixed well with solvent. The solutions were filtered again using no. 1 Whatman filter paper for the removing of unwanted materials particulate. A filtered solution was appropriately further diluted with the elution phase to produce a sample solution for analysis. The amount of PCM and CAF present in the solution sample was estimated using the standard calibration graphs.

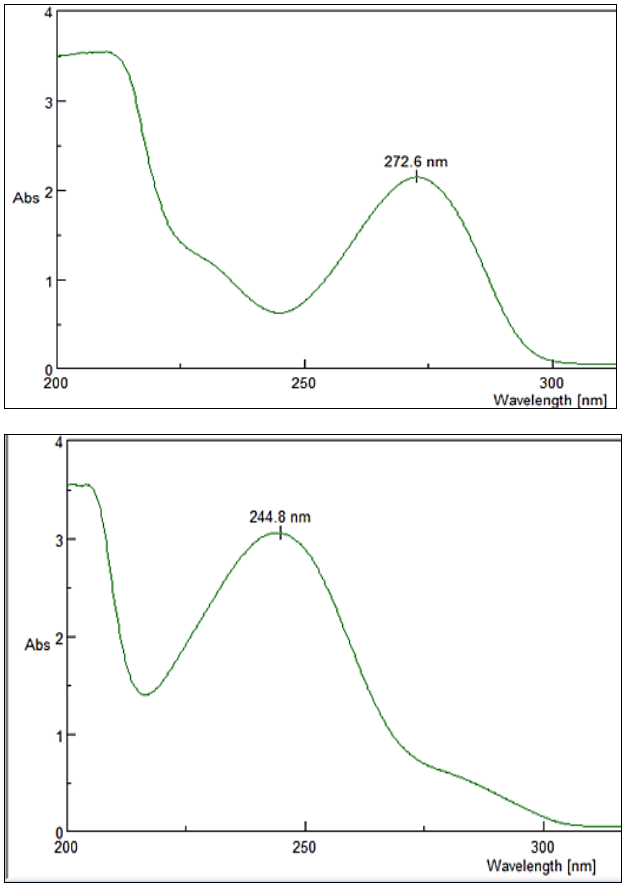

Fig. 1: UV spectra of PCM and CAF.

3. RESULTS AND DISCUSSION:

3.1 Estimation of detection wavelength:

A drugs solution of the 10µg/mL concentration was scanned at the range of 200 to 400nm wavelength. It was observed that PCM and CAF solutions have shown enormous, sharp, and maximum absorbance at 244.8 and 272.6nm wavelength respectively. Therefore, it was selected as the detection wavelength in the analysis. The spectrum study revealed that PCM and CAF solutions were indicating a well - defined λ_{max} at 244.8 and 272.6 nm as clear in (fig. 1).

3.2 Method Development and System Suitability Test:

Various tests were conducted to get reasonable resolution – separation of PCM and CAF using different eluent phases with different ratios of water, organic solvent and buffer. An ideal eluent phase was found to be the mixture of water and methanol. This eluent phase used in ratio (60:40 V/V) gave a good and satisfactory resolution of PCM and CAF. The pH value (4.0) of the eluent phase, increasing or decreasing by ±0.2, did not indicate a worthy change in the analyte retention time. The time of retention using analytical column was estimated at a rate of flow with 1.0mL/min. The volume of injection was 10µL. The retention time of sample and standard for PCM and CAF was well pleased with high resolution in formulating sample. This labour was converging on conditions optimization for the rapid, simple, low cost, and effective analysis, involving a selection of the eluent phase to take out satisfactory results. Solvent strength, solvent type (organic solvent volume fraction in the eluent phase and pH of the mobile phase solution), the wavelength of detection and rate of flow were varied to estimation the Chromatographic conditions which were given the good separation. The optimized of eluent phase conditions was conducted so there no solvent interference and excipients. The entire predicate chromatographic optimum conditions and the notice values of column efficiency, resolution and factor tailing were mentioned in table 1. The chromatogram of PCM, CAF and mixture of drugs applied optimum condition is revealed in (fig. 2).

3.3 Preparation of Calibration graph:

From the standard stock solution, posterior dilutions were done with eluent phase to gain a series of standard solutions have a range of concentration with (0.5 – 25 and 0.1 - 30µg/mL) of drugs. The solutions were injected using injector loop of 10µL and chromatograms were recorded. A graph were plotted by taking a concentration on X-axis and the area under the peak on Y-axis which gave a straight line.

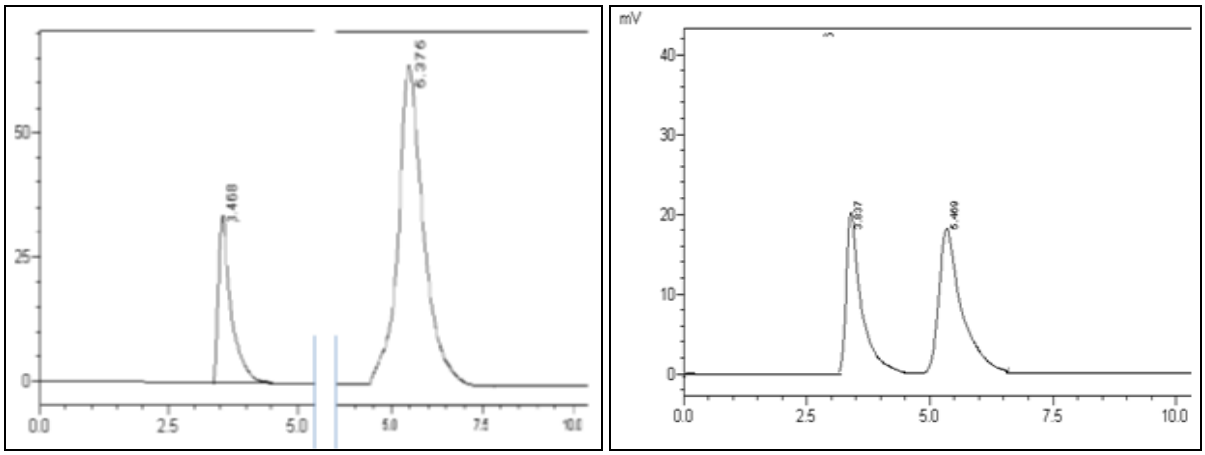

Fig. 2: HPLC chromatograms for PCM and CAF and mixture of drugs.

Table 1: The predicate optimum parameters and system suitability of HPLC method.

Predicate optimum parameters results		
Compassion of eluent phase, water: MOH 60: 40	Column Type, ODS, (250 - 4.6) mm, 5µm	
	Sample Temperature, ambient	
Rate of flow, 1.0 mL/ min.	Column Temperature, 25 ±2 °C	
Volume of injection, 10 µL	Run Time min, 10.00	
Detection wavelength, nm 270	Retention Time min, 3.468 PCM, 5.376 CAF	
System Suitability results		
Parameters of system Suitability	**Results**	**Acceptance criteria**
Retention time	3.468 PCM, 5.376 CAF	
RSD% for area of seven injections of standard drug solution	0.401 PCM, 0.425 CAF	NMT 2.0
Peak talling factor	1.423 PCM, 1.56 CAF	NMT 2.0
Theoretical plates	3798 PCM, 3785 CAF	NLT 2000

3.4 Analytical method validation:

Validation of progress method was conducted as per ICH Q_2 R_1 guideline[24]. Parameters such as accuracy, linearity, precision, specificity, LOD and LOQ, robustness and ruggedness were taken in considering testing for the analytical validation method.

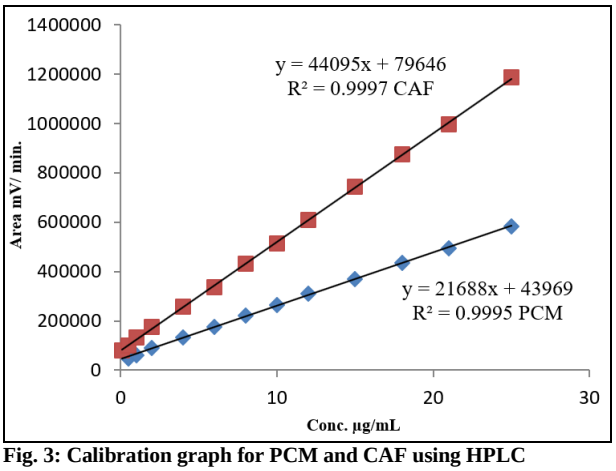

Fig. 3: Calibration graph for PCM and CAF using HPLC

3.5 Linearity and Range:

The proposed RP-HPLC method was shown a good linearity in the concentration range of (0.5 to 25 and 0.1 to 30µg/mL) for PCM, CAF respectively were represented in (fig. 3). The linear equations of the straight lines are y = 21688x + 43969 (R^2 = 0.9995) for PCM and y = 44095x + 79646 (R^2 = 0.9997) for CAF. The results are satisfactory, because there is a significant correlation between concentration of drugs and response factor within the concentration range.

3.6 Precision:

The developed method intraday precision of the was evaluated by analysing PCM and CAF samples of different concentrations three times in the same day and RSD% was estimated.

The precision inter day was estimated through the samples analysing have variable concentrations of PCM and CAF in different three days and RSD% was estimated.

Table 2: parameters validation summery.

Sr. No.	Validation parameters	Results	Standard values
1	Linearity Range	0.5 – 25 PCM, 0.1 - 30 CAF µg/L	-
2	Straight line equation	y = 21688x + 43969 PCM y = 44095x + 79646 CAF	-
3	Correlation Coefficient	0.9995 PCM, 0.9997 CAF	≥ 0.9990
4	Precision (% R.S.D.)		≤ 2.0 % R.S.D.
	Repeatability Intraday Interday	0.314 PCM, 0.265 CAF 0.388 PCM, 0.402 CAF 0.764 PCM, 0.716 CAF	
5	Mean % Recovery	100.28	95 – 105%
6	Specificity	Specific	
7	LOD (µg/mL)	0.01 PCM, 0.005 CAF	-
8	LOQ (µg/mL)	0.033 PCM, 0.016 CAF	-
9	Ruggedness	Complies	≤ 2.0 % R.S.D.
10	Robustness		≤ 2.0 % R.S.D.
	Flow rate change Wavelength change Solution pH change	Complies	

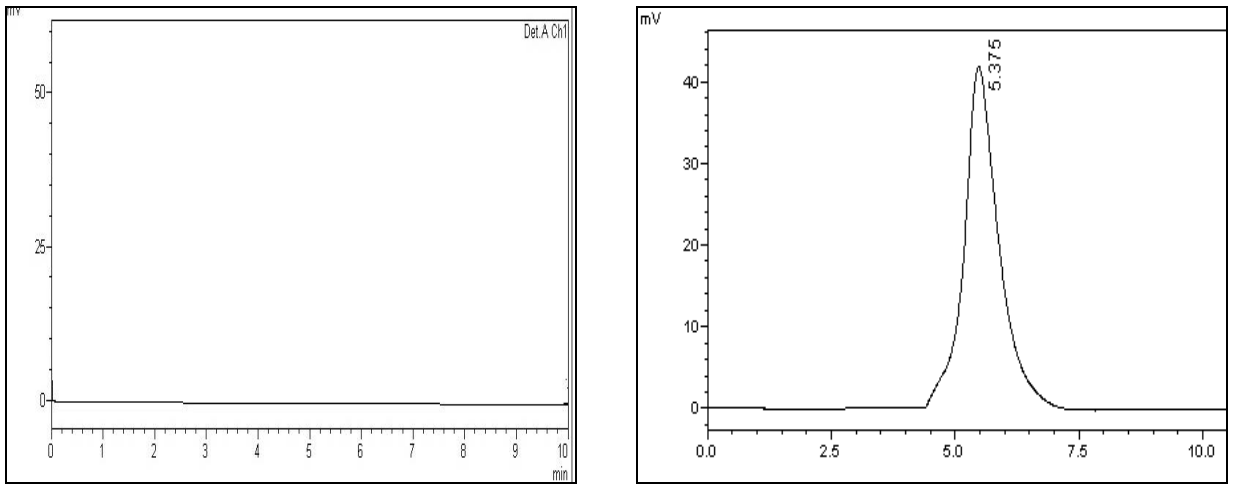

Fig. 4: Specify chromatogram for blank placebo in water: MOH (60:40 v/v) and CAF (10 µg/ L).

Table 3: proposed method accuracy of drugs determination.

PCM µg/mL		% Recovery		CAF µg/mL		% Recovery	
Taken	**Found**			**Taken**	**Found**		
3	2.96	98.67	Mean = 100.54	3	2.95	98.33	Mean = 100.59
5	5.14	102.8	SD = 1.641	5	5.13	102.60	SD = 1.147
7	7.01	100.14	R.S.D. 1.627	7	7.06	100.86	R.S.D. 1.140

Table 4: The ruggedness and robustness results of the proposed method.

Ruggedness results			
	Analyst 1	**Analyst 2**	
Mean % Assay* ± SD	99.87 ± 0.26	98.91 ± 0.21	
% R.S.D.	0.313	0.261	
Robustness results			
Method Robustness Parameters	Mean*	S.D.	%R.S.D.
Flow rate change 1.0 ± 0.1 mL/min.	99.96	0.47	0.47
Mobile phase pH change 4.0 ± 0.2	99.78	0.38	0.38
Detection wavelength change 270 ± 2 nm	100.34	0.21	0.209

*n = 3

Evaluated of Repeatability was conducted by injecting the standard drugs solutions of (5µg/mL) five time in the one day and the RSD% value were calculated. The obtained results are tabulated in table 2. LOD and LOQ were estimated by the gradual dilution for lowest concentration, and 3.3 LOD respectively. The obtained results are tabulated in table 2.

3.7 Accuracy:

This study was carried out to assure the closeness of the test results obtained by the analytical method to the true value[25]. For this method, PCM and CAF were measured at three selected different concentrations within the limits of Beer's law 3, 5, 7µg/mL. The results are tabulated in table 3, which revealed that the suggested method for detection of interesting and quite convenient with respect to the methods and parameters calculated. The recoveries of standard drugs are between 98.33 – 102.80%.

3.8 Specificity:

Specificity, is the analyte ability to unequivocally assess in the presence of other components. Which, may be expected to be present. These components might include degrades, impurities, etc. The placebo solution of eluent phase was injected. The obtained chromatogram revealed there is no inferring peaks at the drugs retention time. The obtained placebo chromatogram was compared with those obtained from the CAF standard solution. The correlation (in terms of t_R and area) was good, which indicate the specificity of the method. The specificity Chromatograms and for the standard CAF were shown in (fig. 4).

3.9 Ruggedness and Robustness:

The proposed method ruggedness was carried out by analysis of aliquots of sample solution (9 PCMµg/mL) by two analysts using same operational and environmental conditions. The method robustness was evaluated by changing the rate of flow by ± 0.1mL/min. (1.1mL/min and 0.9 mL/min), changing the pH by ± 0.2 % (3.8 and 4.2%) for eluent phase and the wavelength detection changing by ± 2 nm (272nm and 268nm). The results obtained are shown in table 4.

4. ANALYTICAL ASSAYS:

Three formulated samples were analyzed for PCM and CAF using a validated high-performance liquid chromatography (HPLC) method with UV detection at 270nm. A 10µL of sample were injected to HPLC analysis under the optimum separation conditions. Eluent phase water: MOH (60: 40 V/V) was delivered at a flow rate of 1.0mL/min with UV detection at 270 nm. The column was Phenomenex C-18 (250mm × 4.6 mm I.D) and 5µm particle size.

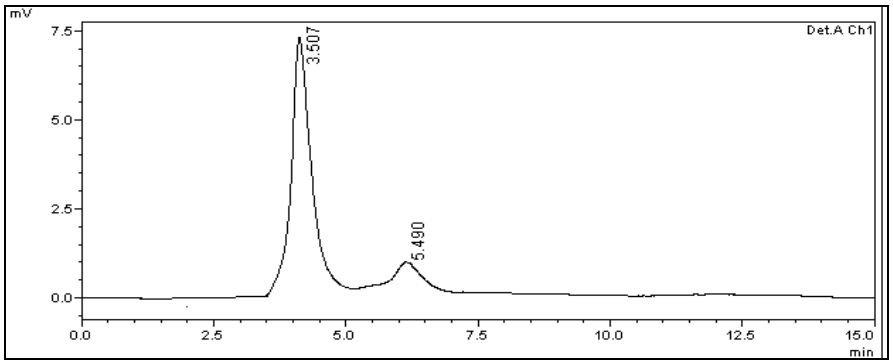

Fig. 5: Separation chromatogram of drugs in formulating sample (Ravmol).

Table 5: Estimated quantity of drugs in different formulating samples.

Name and Company	Drugs type Contain	Claim Label Amount mg/ tab.	Found Mean Amount mg/tab.	% Found Mean Amount	R.S.D n = 3
Piodol Pioneer	PCM	500	501.79	100.36	0.42
Cafergot Turkey	CAF	100	99.57	99.57	0.23
Ravmol India	PCM	325	323.77	99.62	0.35
	CAF	25	24.94	99.76	0.19

Analysis was performed at room temperature (~25ºC) and the total run time was 15 min. The results obtained are tabulated in table 5. Figure 5 is shown the separation chromatograms of the drugs in formulating sample. The recoveries of drugs in samples were between 99.57 – 102.36%.

5. CONCLUSION:

The RP – HPLC validated methods appoint here steady to be accurate, fast, simple, robust, and precise, so it can used in the routine analysis of PCM and CAF as standard and in formulating form.

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7. CONTRIBUTIONS OF AUTHORS:

The authors are all have equally contributed.

8. INTERESTS CONFLICT:

The author has no conflict of interest.

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