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Simultaneous determination of paracetamol and caffeine by RP-HPLC in soft gelatin capsules

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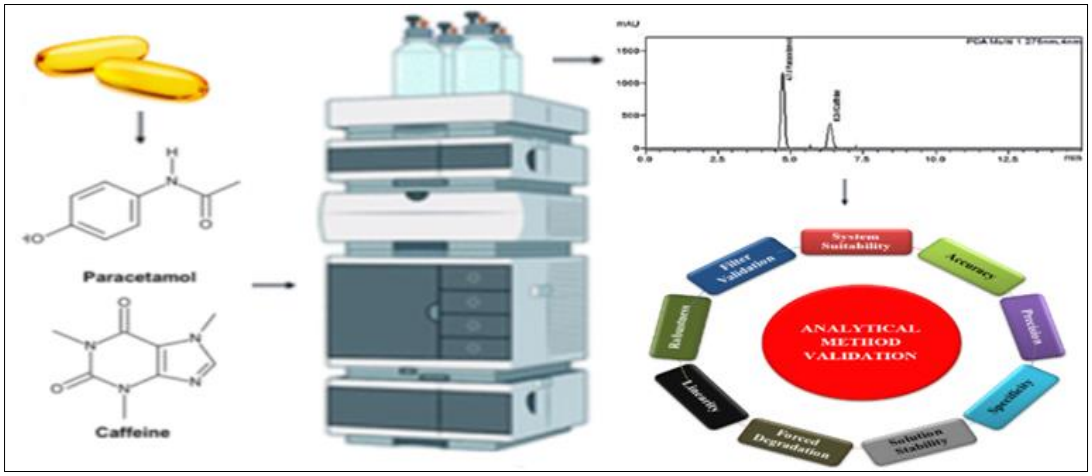
Abstract

A rapid and stability-indicating RP-HPLC method was developed for simultaneous estimation of paracetamol and caffeine in soft gelatin capsules form especially to get some more advantages over other methods already developed for this combination. The method was validated according to ICH guideline with respect to accuracy, precision, specificity, linearity, solution stability, robustness, sensitivity, forced degradation and system suitability. For this, an isocratic condition of mobile phase comprising acetonitrile and phosphate buffer (pH 3.5) in a ratio of 15:85, v/v over YMC C18 column (250 × 4.6 mm, 5 µm) at a flow rate of 1 mL/minute at ambient temperature was maintained. The method showed excellent linear response with correlation coefficient (R2) values of 0.999 and 1.0 for paracetamol and caffeine respectively, which were within the limit of correlation coefficient (R2 > 0.995). The percent recoveries for two drugs were found within the acceptance limit of (98.0–102.0%). % RSD values for repeatability, reproducibility and Intermediate precisions were below 2.0. Forced degradation of the drug product was carried to establishing the stability-indicating property of this method and providing useful information about the degradation pathways, degradation products, and how the quality of a drug substance and drug product changes with time under the influence of various stressing conditions.

Keywords: Simultaneous estimation; Paracetamol; Caffeine; RP-HPLC; Stability-indicating

Graphical abstract



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1. Introduction

Multidrug pharmaceutical formulations for the treatment of pain with a weaker genesis comprise various components that can enhance their pharmacological effectiveness. When it comes to their various mechanisms of action, they occasionally work in unison to increase efficiency. The main metabolic organs are less loaded because each component in the multicomponent preparation is present in smaller amounts than in the monocomponent form of each one. This is because each component interacts with a different metabolic subsystem [1].

Paracetamol is a p-aminophenol derivative having analgesic and antipyretic properties. It is indicated for the treatment of moderate pain, including headache, backache, migraine, rheumatic and muscle pain, and toothache pain also relieves colds, and sore throats and helps reduce temperature. Caffeine has psychoactive and anti-inflammatory properties and acts as an analgesic adjuvant to enhance the efficacy of paracetamol. In pharmaceutical practice, the use of paracetamol with caffeine as an analgesic and antipyretic is well recognized. It is crucial to develop a method for the analysis of caffeine and paracetamol to regulate the amount in pharmaceutical formulations. [2] Rapid and sensitive methods for estimating paracetamol individually and in combination are being studied as an outcome of broad application of paracetamol in various pharmaceutical formulations. Electrochemical techniques, [3-6] chromatographic techniques, [7-10] fluorescence spectroscopic techniques, [11] and spectrophotometric [12-15] techniques were the most recent methods for determination of paracetamol.

For the quantitative estimation of caffeine and paracetamol in various pharmaceutical dosage forms, several HPLC methods [16–17] have been reported. It has been reported that paracetamol and caffeine in formulations can be estimated using spectrophotometric [18] methods. However, a more precise, simple, and widely used HPLC method for determining caffeine and paracetamol simultaneously in soft gelatin capsules has not been published.

2. Chromatographic condition

Analytical conditions were optimized through the HPLC system (Shimadzu Prominence LC-2030C-3D) using mobile phase comprising acetonitrile and phosphate buffer (pH 3.5) in a ratio of 15:85, v/v over YMC C18 column (250 × 4.6 mm, 5 µm) at a flow rate of 1 mL/minute at ambient temperature. The mobile phase was filtered through 0.45µm nylon filters. The volume of injection was fixed at 20 µL and PDA detector with 275 nm wavelength was used for detection.

2.1. Preparation of Mobile Phase

2.1.1. Buffer preparation

6.8 g of potassium dihydrogen orthophosphate was measured in an analytical balance and placed in a 1000 mL beaker, 500 mL of water was added to dissolve, then made up to volume with water, and the pH was adjusted to 3.5 with orthophosphoric acid.

2.1.2. Mobile Phase Preparation

A mixture of acetonitrile and buffer (15:85) v/v was prepared. The above solution was filtered through a 0.45 µm membrane filter and sonicated for 10 minutes.

2.1.3. Diluent

A mixture of acetonitrile and water (30:70 v/v) was prepared. The above solution was filtered through a 0.45µm membrane filter and degassed.

2.2. Standard preparation

2.2.1. Solution A

78.0 mg of caffeine WS/RS was measured in an analytical balance and placed in a 100mL graduated flask and 20 mL of diluent was added. It was firmly shaken and sonicated for 10 minutes and made up to the mark with diluent.

2.2.2. Solution B

60.0 mg of paracetamol WS/RS was measured in an analytical balance and placed in a 100 mL graduated flask, and 10 mL of solution A and 60 mL of diluent was added. It was sonicated for 10 minutes, then made up to its volume with diluent.

3. Validation of the method

According to the ICH guidelines [19], specificity, linearity, accuracy, precision, and system suitability of the proposed method were validated. The accuracy was represented as a percentage of the amount of paracetamol and caffeine recovered in the presence of additives. From six replicate injections of the standard solution, the precision was represented as a percentage RSD of the peak responses for paracetamol and caffeine, respectively. The developed method was validated and then applied to pharmaceutical dosage forms containing paracetamol and caffeine.

3.1. System suitability

By making six replicate injections from the freshly prepared standard solution of caffeine and paracetamol, the system suitability of the proposed approach was established by investigating each solute for its theoretical plates (N), percentage RSD, resolution (R), and tailing factors (T). Before starting the analysis of samples during routine analysis, these system suitability characteristics must be ensured.

3.2. Specificity

The complete separation of caffeine and paracetamol was accomplished in the chromatogram, demonstrating the specificity of the developed method against possible interferences in the presence of blank and placebo. Peak purity index for standards and samples of paracetamol and caffeine were calculated.

3.3. Linearity and range

The linearity of the method was established by performing five standard concentrations from 50.0 % to 150 % of working concentrations as per protocol. The standard solutions were prepared with concentrations of 50 %, 75 %, 100 %, 125 %, and 150 % concerning 100 % working concentration. 6 replicate injections from lower and higher concentrations and 3 replicate injections from remaining concentrations were injected into the HPLC system. Based on the average area obtained with each concentration, a graph is plotted between Area and Concentration.

3.4. Accuracy

By performing analytical recovery experiments using the standard addition method at 50 %, 100 %, and 150 % levels, the accuracy of the developed method and the interference of formulation additives were investigated. Standard and spiked sample solutions with concentrations of 50 %, 100 %, and 150 % were prepared. Following that, the percentage recovery and relative standard deviation were computed.

3.5. Precision

System precision, method precision, and intermediate precision were used to evaluate the precision of the proposed method. For system precision, the standard solution of Paracetamol and Caffeine was prepared and injected in six replicates into HPLC System. For method precision, 6 replicate sample solutions of "Paracetamol-500 mg and Caffeine-65 mg Capsules" were prepared and injected into HPLC System. For intermediate precision, the same batch of "Paracetamol-500 mg and Caffeine-65 mg Capsules" was analyzed in precision with 6 replicate samples, in different labs and using a different Instrument and column on a different day.

3.6. Robustness

The experimental conditions were deliberately changed, and the chromatographic resolution of Paracetamol and Caffeine was evaluated. The robustness of the analytical method was demonstrated with small but deliberate variations in column oven temperature, flow rate, and wavelength. To investigate the effect of flow rate on system suitability parameters, a 20 % change was applied on both sides of the actual flow rate, i.e., from 1.0 to 1.2 mL/min and 0.8 mL/min, while all other conditions were kept constant. For the experiment to study the effect of column oven temperature on system suitability parameters change in column oven temperature of ±2 ºC on either side of the actual temperature was made i.e., from 40 ºC to 38 ºC and 42 ºC while other chromatographic conditions were kept constant. To investigate the effect of wavelength on system suitability parameters, a 2 nm change was applied on both sides of the wavelength, i.e., from 275 nm to 277 nm and 273 nm, while all other conditions were kept constant.

3.7. Filter validation

Filter validation was expressed by centrifuging the sample solution, filtering through 0.45 µm Nylon and 0.45 µm PVDF filters, and assaying the sample without filtration.

3.8. Forced Degradation / Exposure Study

Stress conditions such as Acid Hydrolysis, Base Hydrolysis, Peroxide Oxidation, Heat, Water Hydrolysis, Humidity, and UV Light were generally adopted for sample during the forced degradation study, but conditions were optimized based on the physiochemical properties of the drug substance, available literature, and the amount of degradation achieved during the study. Blank, standard solution, Check standard, Placebo, unstressed sample, and exposure sample were injected into the HPLC system with a photodiode array detector following the test method and the % degradation was calculated.

3.9. Stability of Analytical solutions

The solution stability was demonstrated by injecting standard and sample solution for up to 48 hours. The % RSD of the area of standard and sample solutions was then calculated.

4. Results and discussion

For regular simultaneous determinations of paracetamol and caffeine in soft gelatin capsule dosage forms, the proposed HPLC technique was found to be specific, accurate, precise, and robust in accordance with ICH requirements.

4.1. Optimization of the chromatographic conditions

Herein we put an effort to develop a cost-effective, rapid, and robust reversed phase (RP)-HPLC method with enough data of validation parameters. First, a mixture of acetonitrile and water (15:85) v/v was prepared and used as the mobile phase. Peaks of paracetamol and caffeine were not eluted. So, mobile phase composition was changed for good separation. Then, a mixture of acetonitrile and phosphate buffer (15:85) v/v was prepared and used as the mobile phase.Peaks of paracetamol and caffeine were not eluted in the expected RT. So, another trial was made with a change in the column for good separation. Finally, C18 Column (250 mm X 4.6mm X 5µm) was used for separation. The RT of paracetamol and caffeine meets the described retention time with sharp peaks. So, method was optimized for development.

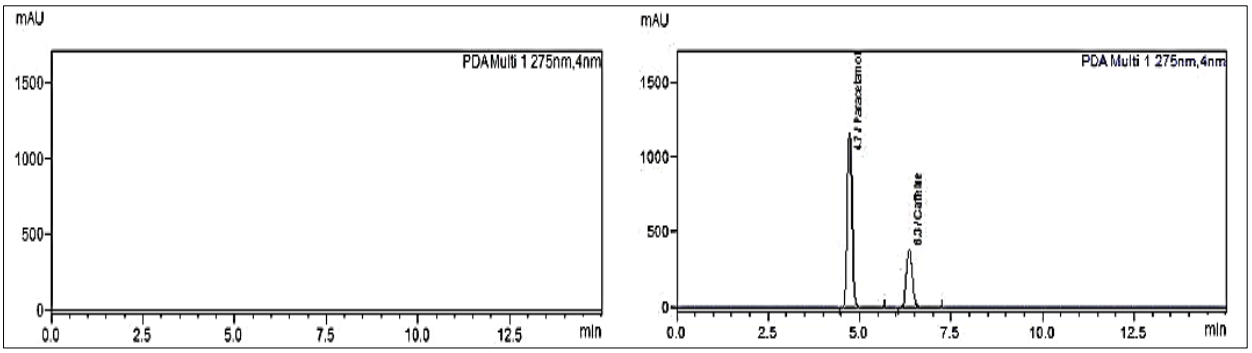

Figure 1 Chromatograms of blank, standard

4.2. Specificity

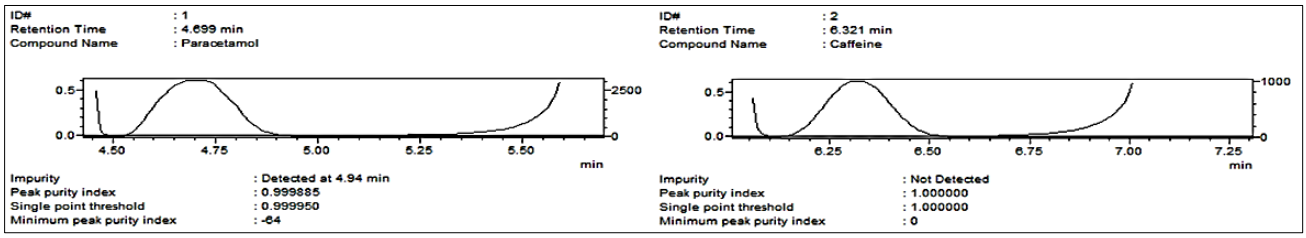

Figure 2 Peak purity of paracetamol and caffeine

The result shows that there is no interference of Blank and placebo peaks at the RT of Paracetamol and Caffeine content in "Paracetamol-500 mg and Caffeine-65 mg Capsules". The peak purity index values of Paracetamol and Caffeine in Paracetamol-500 mg and Caffeine-65 mg Capsules for standard and sample solutions are within the Limit.

4.3. Linearity

The calibration curves were plotted by plotting the absorbance versus the concentration ranges from 50, 75, 100, 125, to 150 % for paracetamol and caffeine. It indicates that, the calibration curves were linear in these concentration ranges with their correlation coefficient values 0.999 and 1.00 for paracetamol and caffeine. Hence it is concluded that the method is linear within the concentration of 50 % to 150 % concerning 100 % working concentration.

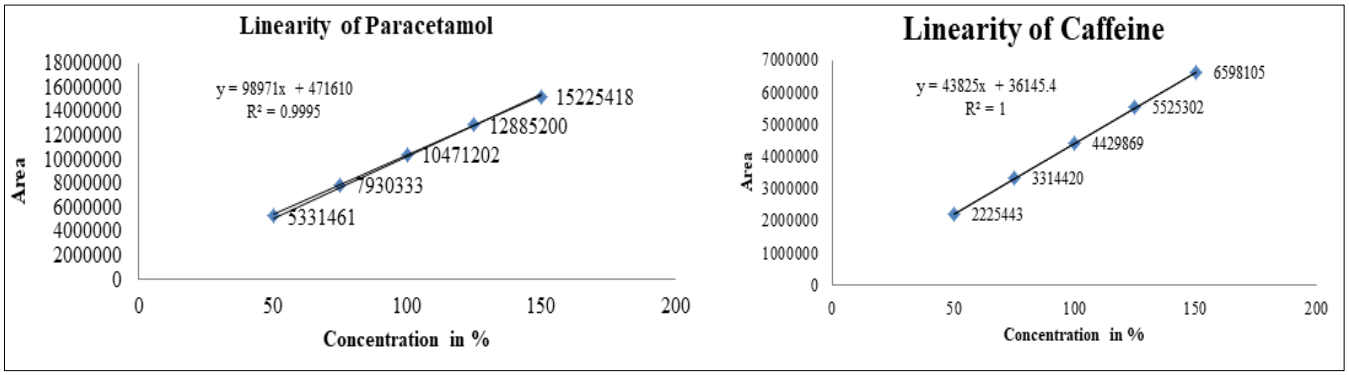

Figure 3 Linearity curve of paracetamol and caffeine

Table 1 Result for Linearity

Parameters	**Paracetamol**		**Caffeine**		**Limits**
Correlation coefficient (r2)	**0.999**		**1.00**		**NLT 0.99**
The % RSD for lower Concentration and higher concentration.	Low (50%)	High (150%)	Low (50%)	High (150%)	NMT 2.0%
	0.2	0.0	0.1	0.1	
% of y-Intercept	4.5		0.8		±5.0

4.4. Accuracy

Analytical recovery experiments were performed using the standard addition method at 50 %, 100 %, and 150 % levels to test the accuracy of the developed method and to investigate the interference of formulation additives. Standard and spiked sample solutions with concentrations of 50 %, 100 %, and 150 % were prepared. The recovery obtained with each concentration level, % RSD of recovery was within the limit. The method was found to be accurate and precise in the range of 50 % to 150 % concerning 100 % working concentration.

Table 2 Summary of Accuracy

Concentration	**Paracetamol**		**Caffeine**	
	% Recovery	RSD (%)	% Recovery	RSD (%)
50%	99.8	0.6	99.4	1.1
100%	99.6	0.0	99.0	0.3
150%	98.6	0.1	101.1	0.3

4.5. Precision

Precision of the developed method was determined based on system precision, method precision, and intermediate precision. Results are presented in Table 3. The % RSD values for system precision, method precision, and intermediate precision were below 2.0. Hence, the method was found to be precise to estimate the amount of Paracetamol and Caffeine.

Table 3 Summary of Precision

PARAMETERS	Tailing Factor		Average % Content		% RSD	
	PARA	CAFF	PARA	CAFF	PARA	CAFF
System Precision	1.0	1.0	101.5	101.3	0.1	0.0
Method Precision	1.0	1.0	101.5	102.5	0.2	0.2
Intermediate precision	1.0	1.0	101.8	103.2	0.9	0.8

4.6. Robustness

The robustness of the analytical method is demonstrated by small variations in the flow rate (0.8 mL/min, 1.0 mL/min, and 1.2 mL/min), column oven temperatures (38 ^{0}C, 40 ^{0}C, and 42 ^{0}C) and wavelengths (273 nm, 275 nm, and 277 nm). The method was found to be robust since the % RSD values for small variations of flow rate, column oven temperatures, and wavelengths were below 2.0.

Table 4 Summary of Robustness

PARAMETERS		Tailing Factor		Average % Content		% RSD	
		PARA	CAFF	PARA	CAFF	PARA	CAFF
Flow rate	0.8 mL/min	1.0	1.0	102.0	101.9	0.1	0.2
	1.0 mL/min	1.0	1.0	101.5	101.3	0.1	0.0
	1.2 mL/min	1.0	1.0	101.9	101.6	0.0	0.5
Column Oven Temperature	38 ºC	1.0	1.0	102.1	102.7	0.1	0.1
	40 ºC	1.0	1.0	101.5	102.0	0.1	0.0
	42 ºC	1.0	1.0	101.7	102.4	0.1	0.1
Wavelength	273 nm	1.0	1.0	102.0	103.1	0.4	0.6
	275 nm	1.0	1.0	101.8	103.1	0.5	0.6
	277 nm	1.0	1.0	101.8	103.0	0.5	0.5

4.7. Forced Degradation

The chromatograms of stressed Blank, Placebo, and sample solutions show that there is no interference of blank, placebo peaks, and degradant peaks at the retention time of Paracetamol and Caffeine. The peak purity index values of standard and sample solutions were within the Limit. Hence, the method was found to be specific to estimate the amount of Paracetamol and Caffeine without the interference of blank, placebo peaks, and degradant peaks, and the method is Stability indicating.

Table 5 Summary of Forced Degradation

DRUG	% OF DEGRADATION						
	ACID	BASE	WATER	OXIDATION	Heat	Humidity	UV Light
Paracetamol	1.4	1.7	1.8	0.6	2.0	1.7	1.6
Caffeine	2.2	1.8	1.7	1.6	1.9	1.7	1.5

4.8. Stability of Analytical solutions

The % RSD of the area of Standard and sample solutions obtained with different time intervals shows that both standard and sample solutions are stable for up to 48 hours.

Table 6 Summary of Analytical solution

Parameters	% RSD		Limit
	Paracetamol	Caffeine	
Standard Solution	0.0	0.1	NMT 2.0
Sample Solution	0.2	0.2	NMT 2.0

4.9. Filter validation

Through the results obtained by centrifugation, 0.45 µm Nylon filter, and 0.45 µm PVDF are well within the limit. So it is recommended to use a centrifuge during regular analysis.

Table 7 Summary of Filter validation

FILTERS	Tailing Factor		Average % Content		% RSD	
	PARA	CAFF	PARA	CAFF	PARA	CAFF
Centrifuge	1.0	1.0	99.6	103.6	1.1	1.1
Nylon 0.45 µm	1.0	1.0	99.6	103.7	1.0	0.7
PVDF 0.45 µm	1.0	1.0	99.4	103.4	0.7	0.6

5. Conclusion

In this study, a validated simple and reliable HPLC–PDA procedure was described for the assay of a multi drug combination consisting of Paracetamol and Caffeine. Both the Paracetamol and Caffeine were successfully resolved and quantified using a Reverse phase C18 column in a relatively short run time. The validation studies show that the proposed HPLC method was accurate, precise, specific and linear in the proposed working range. The good recovery percentage suggests that the excipients have no interference in the determination. The RSD (%) was also less than 2 showing a high degree of precision of the developed method. In terms of flow rate, column oven temperature, and wavelength detection, the developed method was also determined to be robust. Hence the developed method is found to be specific, accurate, precise, robust, and following the specifications of ICH guidelines.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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