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Simultaneous determination of Paracetamol and Caffeine by RP-HPLC method in the tablet dosage form

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Abstract-
The main objective of the current paper is to design a method for the simultaneous determination of paracetamol and caffeine in tablets that are readily available in the local market. We have used the Agilent chromatographic system fitted with a C-18 column for the separation. A mobile phase of methanol-water with composition (40:60 v/v) is used with the flow rate of 1.0 ml min^{-1}. The detection of the peaks occurs at the wavelength 243 nm. The separation of paracetamol and caffeine is achieved without using any buffers or costly chemicals with the mobile phase. The method is linear over the range of 1.0-6.0 mg/100ml with correlation coefficients of 0.9978 for paracetamol and linear over the range of 0.2-1.2 mg/100ml with a correlation coefficient of 0.996 for caffeine. The average retention time for paracetamol and caffeine is found to be 3.37 minutes and 4.67 minutes respectively. The limit of detection for paracetamol and caffeine is 0.273 mg/100ml and 0.0848 mg/100ml respectively. In contrast, the quantitation limit for the paracetamol and caffeine are respectively 0.829 mg/100ml and 0.257 mg/100ml. The precision is expressed as a coefficient of variation and found to be less than 2%. The mean recovery of both the paracetamol and caffeine in the tablet is found to be in the range of 99.6%- 96.0% and 116 %-120% respectively. The content of caffeine is found to be larger than the amount mentioned in the tablet. The proposed method in this paper can be useful in drug validation having two active ingredients i.e. paracetamol and caffeine.

Keywords-Paracetamol, Caffeine, RP-HPLC, Method validation, Tablet

Introduction-

Over the past few years, there has been a large production of drugs having two or more active pharmaceutical ingredients (API). It is due to the effectiveness and efficiency of the drugs with two or more API mixtures. One such combination that is used throughout the world with two active pharmaceutical ingredients is paracetamol and caffeine. The combination of both drugs has been proven effective in the treatment of numerous diseases like fever, cold, headache, backache etc[1].

Paracetamol is a p-aminophenol derivative (N-acetyl-para-aminophenol). It is the most common and versatile drug used as an antipyretic and analgesic medicine for the treatment of fever and pain. Caffeine is 1,3,7-Trimethyl-3,7dihydro-1H-purin-2,6-dion. It is known to be the central nervous stimulant. Caffeine occurs naturally in food like tea, coffee[2,] etc. It is used to treat fatigue, cold, and drowsiness. Since the combination of caffeine and paracetamol as a drug in tablet form

has a worldwide utilization, there is the possibility of adulteration in drug formulation as well[3]. A large number of methods have been suggested for the simultaneous determination of caffeine and paracetamol in the tablet dosage form.

The most common methods are UV- visible spectrophotometric methods[4,5], Flow Injection-Solid Phase Spectrometry[6], NIR[7], HPTLC methods[8,9], Chemometrics methods [10,11], capillary electrophoresis method[12,13] Differential pulse voltammetric[14,15], and HPLC methods[16,17]. Most of the methods reported above are costlier and time-consuming. However, the Reverse-Phase HPLC is one of the most versatile and quick methods for the validation and authentication of drug products in the pharmaceutical sector. It is the most widely and commonly used method. In this paper, the method used is the RP-HPLC method for the validation of the mixture of paracetamol and caffeine in the tablet form.The earlier work done on the separation of caffeine and paracetamol with the same composition of the mobile phase is also given in Table-1. In the present study, we have used different flow rates, peak detection wavelengths, temperature along with the different injection volumes from the earlier studies. It has been observed the retention time is shifted with the sharper signal.

Table-1 Previous RP-HPLC studies using the same mobile phase

S.N.	Solvent composition and flow rate	Peak detection	Retention time	Ref
1	Methanol and water 80:20	254 nm	2.84 min for paracetamol and 3.22 min for caffeine	Dinc E. et al[18]
2	Methanol and water 40:60	264 nm flow rate 0.8 ml/min	2.6 min for paracetamol and 3.5 min for caffeine at temp 35^{0}C	Aminu N., et al[19]
3	Methanol and water 40:60	249 nm and 273 nm dual wavelength detection with a flow rate of 0.5 ml/min	2.6 min for paracetamol and 3.5 min for caffeine at temp 35^{0}C	Prodan M., et al[20].
4	Methanol and water 40:60	243 nm with a flow rate of 1 ml/min	3.37 min for paracetamol and 4.67 min for caffeine at room temp	Present study

Material And Methods -

Methanol (HPLC grade) was procured from Fisher Scientific Pune. The water used is also HPLC grade from Fisher Chemicals pharmaceutical formulation.

The Agilent company HPLC binary system is used for drug analysis. The instrument is equipped with an ultraviolet detector and autosampler. EZ Chrome software is installed on the computer for data acquisition and processing. C18 column (4.6 × 150 mm is used for chromatographic separation having the particle size 5 µm. The mobile phase was prepared with the HPLC grade methanol and water (40:60, v/v). The flow rate of the solvent mixture is maintained during the entire run time of 1 mL/min. The detection of both peaks was carried out at 243 nm. The Injection volume of standard solutions for calibration purposes and sample solutions in each run is 10 µL. All the standards used were of HPLC grade.

Standard solution of paracetamol and caffeine Standard stock solutions of paracetamol and caffeine were prepared by accurately weighing 100 mg of the paracetamol and 20 mg of the caffeine and dissolving them in 100 mL of mobile phase (methanol and water 60:40 v/v). The solutions were then filtered through a 0.25 µ nylon membrane using the filtration assembly. Working standards of paracetamol from the range 1.0 to 6.0 mg/100ml and that of caffeine from 0.2 mg/100ml to 0.6 mg/100 ml were prepared for calibration purposes after dilution of the stock solutions.

Sample solution preparation

Few branded drugs having sufficient shelf lifetimes were purchased from the chemist in the local market. Twenty tablets each containing 500 mg of paracetamol and 25 mg of caffeine were accurately weighed and finely powdered with the help of mortar and pestle. A quantity equivalent to .0326 g of tablet powder was weighed and transferred to a volumetric flask and dissolved in 50 mL of mobile phase i.e. mixture of methanol and water (40:60 V/V.) This sample solution was stirred magnetically for ten minutes. One ml of the above solution is further diluted up to 10 ml containing an equivalent to the ppm paracetamol and 13.6 ppm caffeine. The maxima of the peaks were found at 243 nm for both analytes.

Method validation

Linearity

The linearity of the method is checked by making the standard solution of both analytes. A total of six standard solutions of caffeine and paracetamol were prepared (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 µg/100ml for caffeine and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 µg/100ml for paracetamol.20 µl of each standard solution was injected with the help of HPLC equipment fitted with an autosampler. The results obtained showed that the method is linear for a range of 1.0–6.0 µg/100ml for Paracetamol and for caffeine 0.2–1.2 µg/100 ml with their correlation coefficient (r^2) equal to 0.998 ($r^2 = 1$) and 0.995 respectively. The correlation coefficient (r^2) > .99 indicates the linearity.The equation of a straight line can be written as $y=mx+c$, where m is the slope of the line and c is the intercept of the line on the y axes. These values and other parameters are given for both analytes in Table 2.

Table -2 Various parameters for the paracetamol and caffeine obtained from linearity

Parameter	**Caffeine**	**Paracetamol**
Linearity range	0.2–1.2 mg/100ml	1.0 to 6.0 mg/100ml
Equation	684932x + 4524.3	3368278x+334747.86
Slope(m)	684932	3368278
Intercept(y)	4524.3	334747.86
Correlation coefficient (r^2)	0.995	0.998

Accuracy

The accuracy of a method means the closeness of agreement between the actual value and the measured value. To determine the accuracy of a method, we have performed three successive analyses (n = 3) for different standard solutions having concentrations in the range 2.0 mg/100ml, 4.0 mg/100ml for paracetamol, and 0.4 mg/100ml and 0.8 mg/100 ml for the caffeine. Then the accuracy of the method is calculated in terms of percentage recovery as given by the formula.

$$\text{Percentage recovery } \% = \frac{\text{Recovered conc. of analyte}}{\text{Injected conc. of analyte}} \times 100$$

The results obtained from the determination of accuracy are expressed as percentage recovery. It must be 100± 2% at each conc. range are summarized in Table 3

Table- 3 Result of the accuracy of the method

Name of analyte	injected true conc. (n=3)	found average conc.	% Recovery of analyte
Paracetamol	2.0	1.98	99
	4.0	4.1	102.5
Caffeine	0.4	0.404	101
	0.8	0.785	98.12

Precision-

The precision of the method is defined as the concordance of a series of measurements i.e. closeness in the values which are observed experimentally. Precision is commonly expressed as the relative standard deviation (RSD) by the following relation

RSD = standard deviation(s) / mean of measurement(x)

$$\text{RSD} = \frac{\text{standard deviation(S)}}{\text{mean of measurements(x)}}$$

This is often expressed as a percentage in terms of the coefficient of variance (CV)

$$\text{Coefficient of variation(CV)} = \frac{\text{standard deviation(S)}}{\text{mean of measurements(x)}} \times 100$$

The proposed method is checked for precision from the repeatability of responses after five replicate injections (n = 5) of standard solutions (2.0 mg/100ml for paracetamol and 0.4 mg/100ml for caffeine). The precision was carried out intra-day (same day) and intraday (same day). The precision results are presented in Table 4. In the present method, it comes out to be less than 2. The proposed method is precise.

Table- 4 Determination of precision intra-day and intra-day method

Name of analyte	Injected conc. (n=5)	Intraday			Inter-day			
		Mean conc. recovered	% recovery	Coefficient of variation (CV)	Day	Mean conc. recovered	% recovery	Coefficient of variation (CV)
Paracetamol	2.0	1.93	96.5	1.47	1	1.979	98.94	1.22
	2.0	1.96	98.0			2.029	101.44	
	2.0	1.98	99.0			1.988	99.42	
	2.0	2.01	100.5		2	1.989	99.43	
	2.0	1.96	98.0			1.963	98.14	
						1.976	98.77	
Caffeine	0.4	0.404	101.0	1.76	1	0.398	99.4	1.50
	0.4	0.390	97.5			0.390	97.6	
	0.4	0.409	102.25			0.399	99.8	
	0.4	0.399	99.75		2	0.402	100.6	
	0.4	0.402	100.5			0.409	102.1	
						0.402	100.5	

Detection limit and Limit of quantification-

The detection limit commonly called LD is defined as the lowest amount of analyte in a sample that can be detected. Quantification limit commonly known as LQ is defined as the lowest amount of analyte which can be determined quantitatively with suitable precision and accuracy.

Several approaches have been suggested for the determination of LD and LQ. The most common method is to use the standard deviation and the slope of the calibration curve.

$$\text{Detection limit (LD)} = \frac{3.3 \times \text{standard deviation}}{\text{Slope of the calibration curve}}$$

LD values for paracetamol and caffeine are 0.273 mg/100ml and 0.0848 mg/100 ml respectively.

$$\text{Detection limit (LQ)} = \frac{10 \times \text{standard deviation}}{\text{Slope of the calibration curve}}$$

LQ values for paracetamol and caffeine are 0.829 mg/100ml and 0.257 mg/100ml respectively.

Analysis of dosage form

Twenty tablets weighed accurately and then crushed in a mortar with a pestle. A quantity of about 32.6 mg was dissolved in the mobile phase. The following data were obtained after HPLC analysis of the tablet as per details given in table 5

Table-5 Analysis of tablets containing caffeine and paracetamol

Sample	Paracetamol claimed in tablet	Amount found	Assay (%)	Caffeine claimed in tablet	Amount found	Assay (%)
Sample -1	500 mg	498	99.6	25 mg	29 mg	116
Sample-2	500 mg	480	96.0	25 mg	30 mg	120

Discussion*:*

As per the guidelines issued by the Indian Pharmacopoeia (I.P), the amount of active ingredient in the tablet should contain not less than 90% and not more than 110% of the labelled content. It means the limiting amount of paracetamol must be from 495 mg to 550 mg and 22 mg to 30 mg of caffeine in the combined dosage form of tablet. In our study of the drug containing these active ingredients as per the details given in table 5. Paracetamol has come out to be in the range of 99.6%-99.0% while the amount of caffeine is in the range of 120 %-116%. The amount of Caffeine is slightly more than the prescribed limits as per the guidelines of Indian Pharmacopeia.

Conclusion

From the above discussion, it is clear that the present method can be used for qualitative and quantitative analysis of paracetamol and caffeine together in the drug formulation without the use of buffers. It is quite easy to use and less time-consuming. It is the amount of caffeine that comes out to be slightly greater than the labelled amount in the market tablet.

Conflicts of interest

None of the conflicts of interest.

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