ISSN: 2573-962X

Research Article

Journal of Pharmaceutical Research

Simultaneous Equation Method for the Estimation of Caffeine and Pioglitazone HCL by UV-Visible Spectrophotometry

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Submitted: 2023, June 09; **Accepted:** 2023, June 29; **Published:** 2023, July 18

Citation: Shaikh, M, A., Agnihotri, J., Saba, K. (2023). Simultaneous Equation Method for the Estimation of Caffeine and Pioglitazone HCL by UV-Visible Spectrophotometry. *J Pharmaceut Res, 8*(2), 229-237.

Abstract

A simple, rapid, accurate, economical and precise UV/VIS method has been developed and validated. Choices of a common solvent were essential so various solvent ranges including methanol, ethanol, acetonitrile, and 0.1 N HCL, and various concentrations ranges of various buffers were analyzed. Hence 0.1 N HCL was selected as a solvent for the proposed method. Caffeine and Pioglitazone HCL showed maximum absorbance at 273 and 220 nm respectively. Both drugs obey Beer Lambert's law in the concentration range of 3-18 µg/mL for Caffeine and Pioglitazone HCL respectively. The LOD and LOQ were found to be 0.5476 µg/mL and 1.6594 µg/mL for Caffeine respectively. For Pioglitazone HCL the LOD and LOQ values were found to be 0.6111 µg/mL and 1.8519 µg/mL respectively. The method was quantitatively evaluated in terms of linearity, precision, precision, LOD, LOQ and recovery. The method is simple, convenient and suitable for the analysis of Caffeine and Pioglitazone HCL in bulk drugs.

Keywords: Simultaneous method, Caffeine, Pioglitazone HCL, spectrophotometric, UV method, Validation.

1. Introduction

Caffeine (CAF) is chemically 1,3,7-Trimethylpurine-2,6-dione (Figure 1). Caffeine increases intracellular concentrations of cyclic adenosine monophosphate (cAMP) by inhibiting phosphodiesterase enzymes in skeletal muscle and adipose tissues [01].

Pioglitazone HCL (PIO) is chemically (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione (figure 2). In persons with Type 2 diabetes, pioglitazone improves glycemic management by increasing insulin sensitivity through its activity at PPAR gamma 1 and PPAR gamma 2. It affects lipid metabolism through action at PPAR alpha [02].

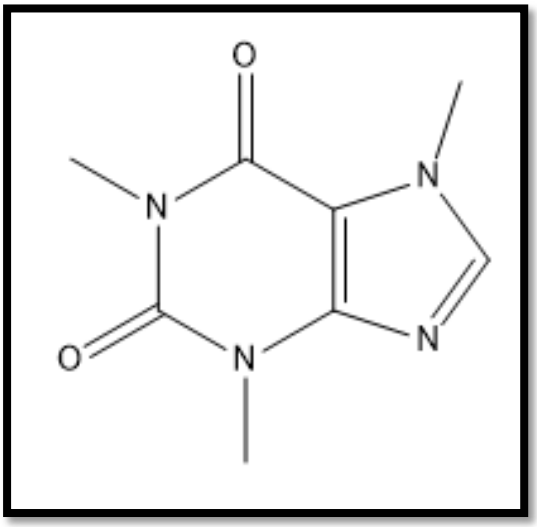

Figure 1: Structure of Caffeine

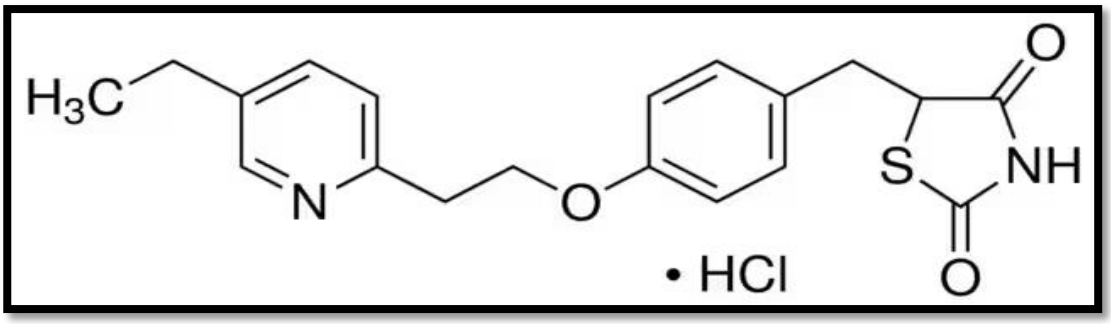

Figure 2: Structure of Pioglitazone HCL

Literature survey publicized that certain UV (2–9), HPLC (1, 10–16), HPTLC (17), and LC-MS (18) methods were reported for the estimation of these drugs individually or combined with other drugs. On the other hand, simultaneous equation (SE) or Vierordt's method was not reported for this new combination. Typically, the simultaneous equation (SE) or Vierordt's approach is used to estimate drug combinations that contain two or more pharmaceuticals in the combined dosage form. Comparing this method to other UV technologies, the technical difficulties are quite little. To ensure the safety and effectiveness of this chosen combination, an effort has been made to design an easy-to-use, reproducible SE approach. For the simultaneous determination of CAF and PIO in pure and pharmaceutical dosage forms, this devised approach was fully validated and successfully used.

2. Materials and methods:

2.1. Apparatus: Shimadzu 1650 UV-VIS double beam spectrophotometer with UV probe software was used. Absorbance measurements were recorded with a pair of 1 cm matched quartz cells.

2.2. Chemicals and Reagents: Caffeine and pioglitazone HCL were kindly supplied by Medley Pharmaceutical and FDC respectively (Mumbai, India) and analytical grade Hydrochloric acid (Vishal chem) was used.

2.3. Preparations of Standard Stock Solutions: Standard stock solution of Caffeine (1000 µg/mL) was prepared by dissolving 100mg of Caffeine in 60 mL of 0.1 N HCL. The resulting solution was sonicated for 10 minutes and the final volume was adjusted to 100 mL with 0.1 N HCL. From this standard stock solution, 1 mL was withdrawn and diluted to 10 mL using the same solvent to get a working standard solution of 10 µg/mL. Similarly, a standard stock solution of Pioglitazone HCL (1000 µg/mL) was prepared by dissolving 1000 mg of Pioglitazone HCL in 60 mL of 0.1 N HCL. The resulting solution was sonicated for 10 minutes and the final volume was adjusted to 100 mL with 0.1 N HCL. This solution was further diluted to get a working standard solution of 10 µg/mL.

2.4. Simultaneous Equation Method Development: Working solutions of both drugs were scanned in the UV range of 200–400 nm. The overlay spectra of both drugs were recorded (Figure 3). From overlain spectra, wavelengths 273 nm (of CAF) and 220 nm (of PIO) were selected for analysis of both drugs using a simultaneous equation method (273 nm for CAF and 220 nm for PIO). Consequently, it may be possible to determine both drugs by the technique of from method or simultaneous equation method (19–22).

Six standard solutions having concentrations of 3,6,9,12,15 and 18 µg/mL for CAF and 3,6,9,12,15 and 18 µg/mL for PIO were prepared in 0.1 N HCL and their corresponding absorbance was measured at 273 nm and 220 nm. The concentration of drugs x (CAF) and y (PIO) in sample solutions were determined by the SE method using the following formula:

A1 = ax1 b Cx + ay1 b Cy equation 1

A2 = ax2 b Cx + ay2 b Cy equation 2

where Cx and Cy are the concentration of CAF and PIO, A1 and A2 are the absorbance of sample solution at 273 nm and 220 nm, respectively, ax1 and ax2 are absorptivities of CAF at 273 nm and 220 nm, ay1 and ay2 are absorptivities of PIO at 273 nm and 220 nm, respectively [03].

The absorptivity value of CAF and PIO from each solution was calculated using the following formula and the results were presented in Tables 1 and 2:

Developed method was validated as per ICH guidelines [04].

3. Results and Discussion

3.1. Specificity: Specificity and selectivity are both terms to describe the extent to which other substances interfere with the determination of a substance according to a given analytical procedure. Such other substances might include impurities, degradation products, related substances, matrix or other components present in the operating environment. Specificity is typically used to describe the ultimate state, measuring unequivocally a desired analyte. Selectivity is a relative term to describe to which extent particular analytes in mixtures or matrices can be measured without interferences from other components with similar behavior.

The specificity of the method was dogged by measuring the absorbance of CAF and PIO individually at 273 nm and 220 nm against the blank and synthetic excipients and their absorbance was compared with the blank and synthetic excipients. No interference was observed at 273 nm and 220 nm indicating that the method is specific.

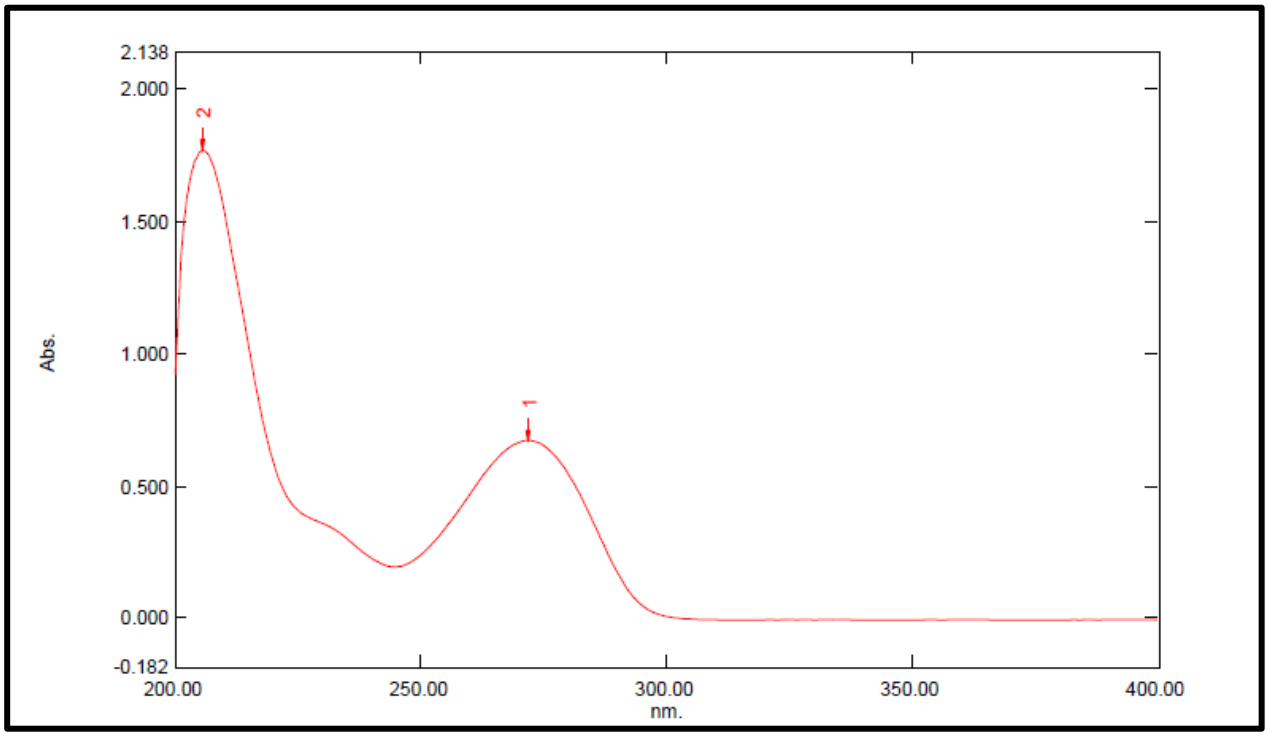

Figure 3: UV Spectrum of Caffeine

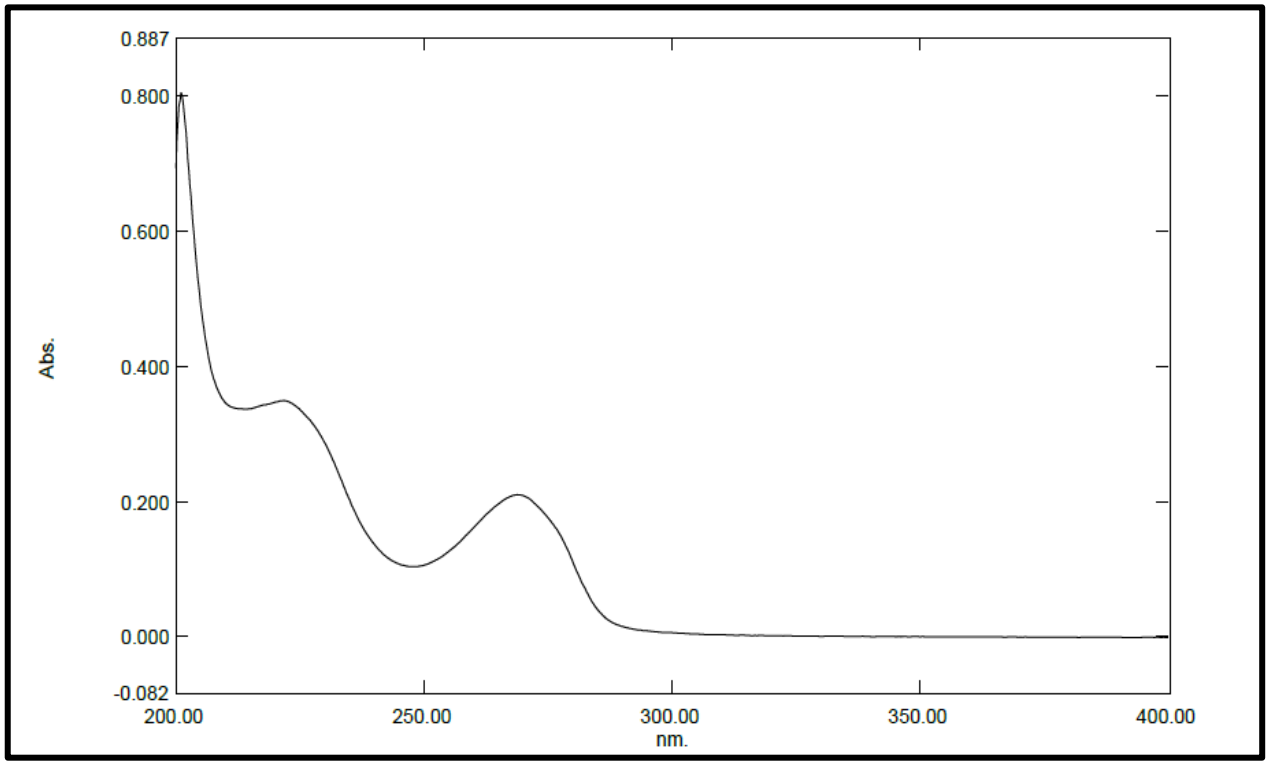

Figure 4: UV Spectrum of Pioglitazone HCL

3.2. Linearity: The calibration curves (Figures 4 and 5) were constructed by plotting the absorbance versus the concentration ranges from 3,6,9,12,15 and 18 µg/mL and 3,6,9,12,15 and 18 µg/mL for CAF and PIO. It was found that the calibration curves were linear in these concentration ranges with their correlation coefficient values 0.9993 for CAF and 0.9991 for PIO. Results revealed that good correlation exists between the concentration of the sample and their absorbance.

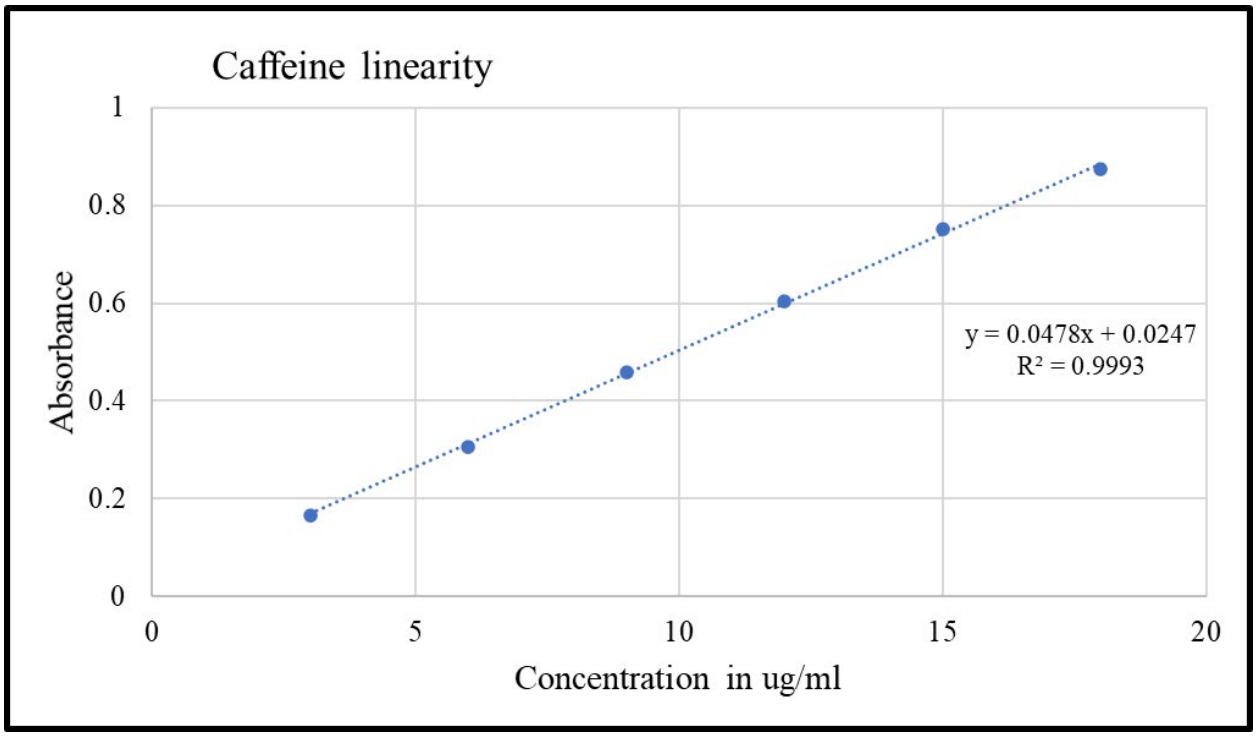

Figure 5: Linearity of Caffeine

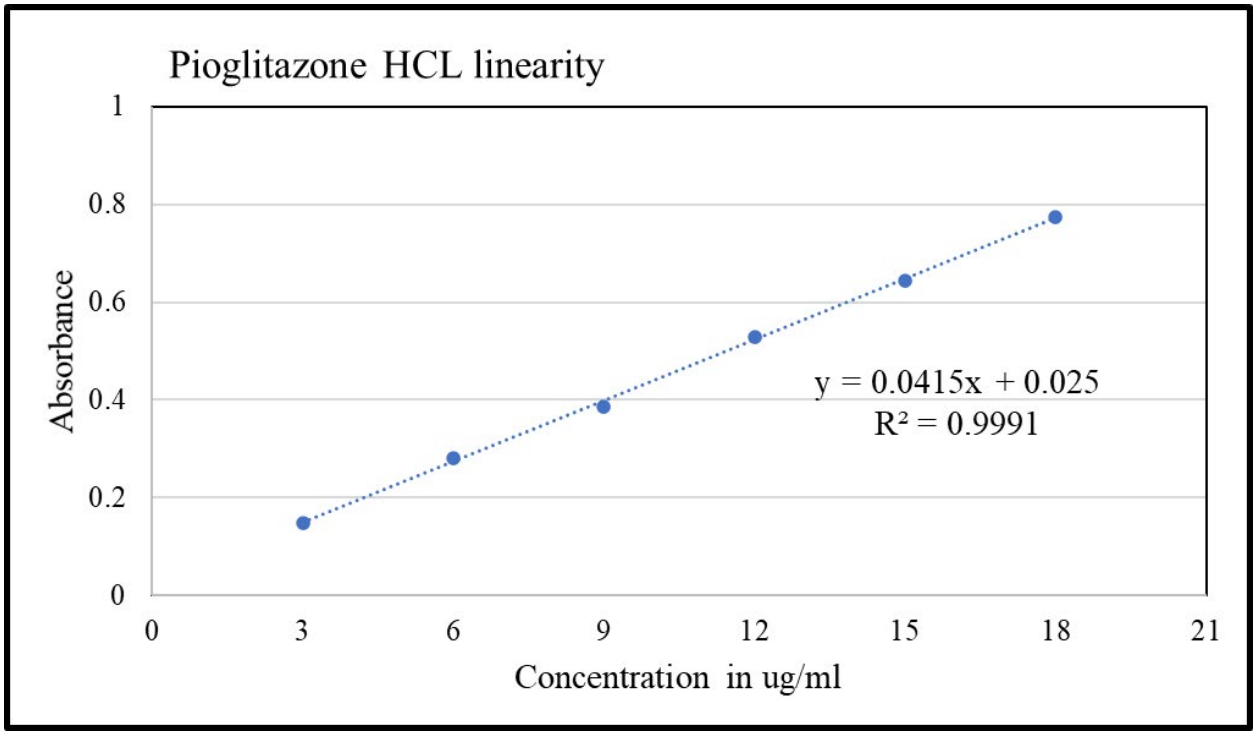

Figure 6: Linearity of Pioglitazone HCL

Optical characteristics

Parameter	Caffeine	Pioglitazone HCL
Wavelength	273 nm	220 nm
Equation	y = 0.0478x + 0.0247	y = 0.0415x + 0.025
Slope	0.0478	0.0415
Intercept	0.0247	0.025
Correlation Coefficient (R2)	0.9993	0.9991
Range	3-18	3-18
LOD	0.5476172417	0.6111595186
LOQ	1.659446187	1.851998541

Table 1: Optical characteristics data

3.4. Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions. Precision can be considered at three levels: repeatability, intermediate precision and reproducibility.

3.4.1 Repeatability: This study was performed with a minimum of six replicate measurements of absorbance of sample solution on the same day.

Theoretical Concentration (µg/mL)	CAF			PIO		
	Measured Absorbance	Standard Deviation	% RSD	Measured Absorbance	Standard Deviation	% RSD
12	0.5955	0.0069	1.1670	0.519	0.0052	1.0048

Table 2: Inter day precision data of CAF and PIO (n=6)

3.4.2 Intermediate Precision: Intermediate precision was performed by measuring the absorbance of the sample solution on three different days and on the same day.

Theoretical Concentration (µg/mL)	CAF			PIO		
	Measured Absorbance	Standard Deviation	% RSD	Measured Absorbance	Standard Deviation	% RSD
3	0.1613	0.0030	1.8936	0.1316	0.0011	0.8769
12	0.6083	0.0035	0.5772	0.5243	0.0030	0.5826
18	0.8606	0.0051	0.5962	0.7763	0.0015	0.1967

Table 3: Inter day precision data of CAF and PIO (n=3)

Theoretical Concentration (µg/mL)	CAF			PIO		
	Measured Absorbance	Standard Deviation	% RSD	Measured Absorbance	Standard Deviation	% RSD
3	0.1696	0.00305	1.8006	0.133	0.002	1.5037
12	0.62	0.001	0.1612	0.5163	0.0011	0.2236
18	0.872	0.0017	0.1986	0.7876	0.0037	0.4806

Table 4: Intraday precision data of CAF and PIO (n=3)

3.4.3 Reproducibility: The method's reproducibility was checked in three different analysts and the results were compared.

Sr No	Theoretical Concentration (µg/mL)	CAF			PIO		
		Measured Absorbance	Standard Deviation	% RSD	Measured Absorbance	Standard Deviation	% RSD
Analyst 1	12	0.595	0.001	0.1680	0.5116	0.0037	0.7399
Analyst 2	12	0.5926	0.0005	0.0974	0.515	0.0017	0.3363
Analyst 3	12	0.602	0.001	0.1661	0.5136	0.0011	0.2247

Table 5: Reproducibility data of CAF and PIO (n=3)

The low % RSD (<2%) for CAF and PIO indicated that the method is precise.

3.5. Robustness: The robustness of the method was determined by changing the wavelength ±1 nm from 273 nm to 272 and 274 nm for CAF and 220 to 219 and 220 for PIO and the results were offered in Table 5.

Conc in ug/ml	Mean Absorbance	SD	%RSD
12 (272 nm)	0.6003	0.009073771726	1.5114
12 (274 nm)	0.5916	0.0092	1.5704

Table 6: Robustness data of CAF (n=3)

Conc in ug/ml	Mean Absorbance	SD	%RSD
12 (219 nm)	0.504	0.0026	0.5249
12 (221 nm)	0.508	0.0026	0.5208

Table 7: Robustness data of PIO (n=3)

The % RSD value calculated from the robustness study was found to be less than 2% for CAF and PIO, indicating that the method is robust.

3.6. Accuracy

Set	Sample Conc. (µg/ml)	Standard added (µg/ml)	Mean of Recovery	SD	RSD
80	12	7.2	100.91	1.0476	1.0381
100	12	9	100.17	0.355	0.3544
120	12	10.8	101.36	0.6984	0.689

Table 7: Accuracy data of CAF (n=3)

Set	Sample Conc. (µg/ml)	Standard added (µg/ml)	Mean of Recovery	SD	RSD
80	12	7.2	98.05	1.1547	1.1776
100	12	9	100.30	0.8565	0.8539
120	12	10.8	100.39	1.1150	1.1105

Table 8: Accuracy data of PIO (n=3)

3.7. Limit of Detection (LOD) and Limit of Quantification (LOQ): The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. An analytical procedure's quantitation limit should not exceed the reporting threshold. The quantitation limit is a parameter used for quantitative assays for low levels of compounds in sample matrices, and, particularly, is used for the determination of impurities and/or degradation products.

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration graph. LOD and LOQ for CAF were found to be 0.54 ug/mL and 1.65 ug/mL for PIO 0.61 ug/mL and 1.85 ug/mL respectively.

3.8. Stability: The stability of the standard and sample solutions was checked for three days at room temperature and the absorbance was measured on each day. The amount of drug present in the sample solution was calculated and the results confirmed that the sample solution is stable for three days without any degradation at room temperature.

3.8. Application of Developed Method to Marketed Dosage Forms: 20 tablets were weighed and flattened into powder. Powder weight equivalent to 150 mg of CAF and 15 mg of PIO was transferred into a 100 mL volumetric flask. 50 mL of solvent (0.1 N HCl) was added and sonicated for 20 minutes. Then the final volume was diluted up to the mark with the solvent (0.1 N HCl) and filtered. 2 mL of the above filtrate was transferred into a 25 mL volumetric flask, and the final volume was adjusted up to the mark with the same solvent to get sample solution. The absorbance of the resulting solution was measured at 273 and 220 nm and the amount of CAF and PIO present in each tablet was found to be 148.4 mg and 14.6 mg, respectively. The assay results are described in Table 9.

Label claim (µg/ml)		Conc. Found (µg/ml)	
CAF	PIO	CAF	PIO
150	15	148.4	14.6

Table 9: Assay results

Finally, the developed new simple simultaneous equation method was applied successfully to the marketed tablet dosage form. The assay results indicated that this method can be effectively used to estimate both drugs in the combined dosage form.

4. Conclusion

The developed simultaneous equation method is simple, precise, specific, and accurate. Statistical analysis proved that the method was repeatable and selective for the simultaneous estimation of CAF and PIO in pure and pharmaceutical dosage forms without any interference from the excipients. This new simple method can be used routinely for the estimation of these drugs.

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