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Original Research Article

Analytical method development and validation of UV-visible spectrophotometric method for the estimation of CNS stimulant drug in bulk

Saba Khan [1,*], Mohd Anas Shaikh [1], Nihal Shaikh [1], Jaya Agnihotri [1]

[1] *Dept. of Pharmaceutics, H K College of Pharmacy, Mumbai University, Mumbai, Maharashtra, India*

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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 showed maximum absorbance at 273nm.Drug obey Beer Lambert's law in the concentration range of 3-18 μg/mL for Caffeine. The LOD and LOQ were found to be 0.5476 μg/mL and 1.6594 μg/mL for Caffeine. 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 in bulk drugs.

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. [1]

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.

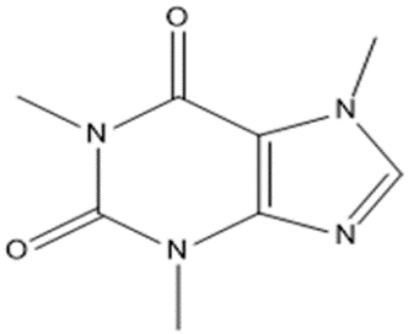

Fig. 1: Structure of caffeine

2.2. Chemicals and reagents

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

* *Corresponding author*.
E-mail address: khansabawahid@gmail.com (S. Khan).

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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.

Developed method was validated as per ICH[2] guidelines.

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 at 273 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 indicating that the method is specific.[3,4]

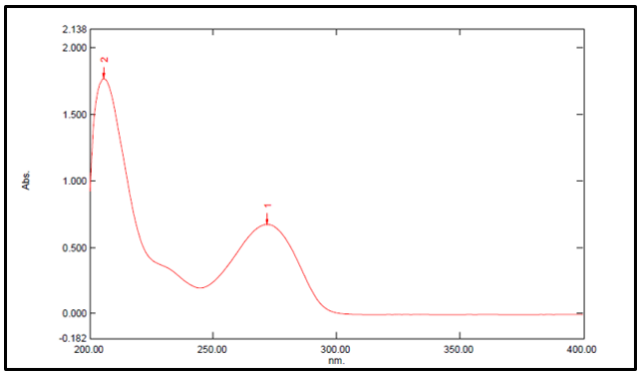

Fig. 2: UV spectrum of caffeine

3.2. Linearity

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

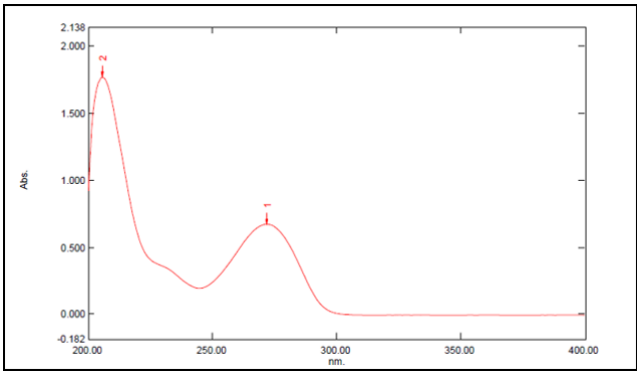

Fig. 3: Linearity of caffeine

3.3. Optical

3.3.1. Characteristics

Table 1: Optical characteristics data

Parameter	Caffeine
Wavelength	273 nm
Equation	y = 0.0478x + 0.0247
Slope	0.0478
Intercept	0.0247
Correlation Coefficient (R2)	0.9993
Range	3-18
LOD	0.5476172417
LOQ	1.659446187

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.[7,8]

3.4.1. Repeatability

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

Table 2: Interday precision data of CAF (n=6)

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

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.

Table 3: Interday precision data of CAF (n=3)

Theoretical Concentration (μg/mL)	CAF		
	Measured Absorbance	Standard Deviation	% RSD
3	0.1613	0.0030	1.8936
12	0.6083	0.0035	0.5772
18	0.8606	0.0051	0.5962

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

Theoretical Concentration (μg/mL)	CAF		
	Measured Absorbance	Standard Deviation	% RSD
3	0.1696	0.00305	1.8006
12	0.62	0.001	0.1612
18	0.872	0.0017	0.1986

3.4.3. Reproducibility

The method's reproducibility was checked in three different analysts and the results were compared.[9,10]

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

Sr. No	Theoretical Concentration (μg/mL)	CAF		
		Measured Absorbance	Standard Deviation	% RSD
Analyst 1	12	0.595	0.001	0.1680
Analyst 2	12	0.5926	0.0005	0.0974
Analyst 3	12	0.602	0.001	0.1661

The low % RSD (<2%) for CAF 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 the results were offered in Table 6.

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

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

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

3.6. Accuracy

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	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

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.

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.

3.9. Application of developed method to marketed dosage Forms

20 tablets were weighed and flattened into powder. Powder weight equivalent to 150 mg of CAF 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 the amount of CAF present in each tablet was found to be 149.2 mg, respectively. The assay results are described in Table 8.[11,12]

Finally, the developed new simple 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.

Table 8: Assay results

Label claim (mg)	Conc. Found (mg)
150	149.2

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 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.

5. Source of Funding

None.

6. Conflict of Interest

None.

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Author biography

Saba Khan, Research Student https://orcid.org/0000-0002-3933-8776

Mohd Anas Shaikh, Research Student https://orcid.org/0009-0001-0796-6971

Nihal Shaikh, Assistant Professor

Jaya Agnihotri, HOD

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