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Research Article

Method Development of Caffeine

Caffeine in Tablet Dosage Form: Analytical Method Development and Validation by Using U.V. Spectroscopy

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Abstract: Caffeine (1,3,7-trimethylpurine 2,6 dione) is a CNS stimulant. Caffeine is used as a drug having drug-addicted properties having a U.V. cutoff in the range of 272 nm to 297 nm in the U.V. spectrum. In this paper, we took a tablet with Caffeine as a single dose in solid dosage form with excipients that do not have a sharp peak in the U.V. spectrum range from 200 nm to 400 nm. The solvent used as a vehicle is a simple polar solvent with a U.V. cutoff at around 189nm to 191 nm. The U.V. spectrophotometer that we used is of Shimdzu having a double beam, which has two provisions to keep reference and sample at a time to complete sample analysis. Firstly we make a calibration curve of Caffeine with a polar solvent. For the research, development, and production of medicines, analytical technique related to creation and validation are essential. Therefore, in this study, a readily reproducible, straightforward, and quick technique of U.V. spectrophotometric quantitative measurement of Caffeine in the form of tablets is adopted. According to ICH criteria, the parameters to be examined include linearity, specificity, accuracy, precision, and robustness. Purified/deionized water was used as the diluent for the drug quantification at the wavelength of 248.5 nm. Beer-law Lambert's applies to Caffeine at concentrations between 10 and 50 g/ml. Recovery was used to test the method's accuracy, and the results ranged from 99.46 to 100.67%. This approach was used for routine analysis of Caffeine in tablet dosage form.

Keywords: U.V. Spectroscopy, Caffeine, Method Development, Method Validation, Caffeine Tablet

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1. INTRODUCTION

The research, validation, and transfer of analytical techniques must be integral parts of every pharmaceutical development effort. The development and validation processes used with drug products and heterocyclic compounds will be the main emphasis of this technical brief[1-4]. However, they need more consideration to be given to them in light of their potential to improve overall development cost and time efficiencies since they are often seen as routine analyses. Analytical techniques aim to determine the identification, purity, physical properties, as well as potency of the pharmaceuticals we Consume[5-8]. Drug testing against standards is supported by methods established for production, quality release, and long-term stability research[9-13]. Methods may also be employed to assess a drug's efficacy or conduct studies on safety and characterization. According to the International Conference on Harmonization (ICH), the most frequently used analysis techniques are (i) parameterization testing of the active ingredient in samples of API or drug product or other chosen element(s), (ii) empirical tests for impurities' content limit tests for the control of chemical impurities, (iii) limit tests for the regulation of contaminants, and (iv) identification tests. The experimental setup goes hand in hand with the development of the medicinal product. If there are time, money, or productivity limitations, the step-in-the-process methodology notion is essential. The objective and strategy should be suitable for developing a prescription medicine. Early drug development methods may focus on the behaviour of the API. They should be able to assist in pre-clinical safety evaluations, pre-formulation research, and stability testing on prototype products—knowledge of APIs and pharmaceuticals. The techniques must be reliable and simple while also adhering to regulatory requirements. The analytical techniques are improved and broadened as drug development advances, based on increased[14,15].

1.1 Elements of Validation

The confirmation of an analytical procedure shows a measurement or characterization's scientific validity. It is necessary for varying degrees at different stages of the regulatory submission procedure. The process of validation demonstrates where an investigative technique measures the appropriate item, in the appropriate amount, within the appropriate range for the intended sampling. It helps the analyst ascertain the procedure's performance limits and grasp its behaviour.

1.2 Caffeine

Caffeine is a central nervous system (CNS) stimulant belonging to the methylxanthine family. The most widely utilized psychoactive drug globally is this one. Contrary to many other psychoactive chemicals, it is accepted and unrestricted almost everywhere in the world. Some recognized mechanisms of action can explain the effects of Caffeine. The most notable is that it delays the onset of sleepiness brought on by adenosine by reversibly blocking adenosine's action on its receptor. Additionally, Caffeine stimulates a few areas of the autonomic nervous system. The guanine and adenine nucleotides of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have molecular resemblances with the caffeine methylxanthine alkaloid. This purine is white, bitter, and crystalline. Many South American and East Asian natural plants have it in their nuts, seeds, or leaves, and it aids in defending them against predatory insects and halting the germination of neighbouring seeds. The most common source of Caffeine is indeed the coffee bean. Drinks with Caffeine are used to minimize or prevent tiredness and improve performance. The plant matter is steeped in water to create these beverages' amount of Caffeine, a process called infusion. Coffee, tea, and cola are three common beverages containing Caffeine; in 2005, 90% of North American adults reported daily caffeine consumption.

1.3 Chemistry of Caffeine

A white, odourless powder with a bitter taste that is pure anhydrous Caffeine (Figure 1) has a melting point of 235-238 °C. At room temperature, Caffeine is only a little soluble in water (2 g/100 mL), while it is highly soluble in boiling water (66 g/100 mL). Additionally, it is only slightly soluble in ethanol (1.5 g/100 mL). It requires strong acid to protonate it because it is only weakly basic (pKa = 0.6). Since Caffeine lacks any stereogenic centres, it is categorized as an achiral molecule. A pyrimidinedione and an imidazole are two fused rings that make up the xanthine core of Caffeine. The pyrimidinedione consists of two amide functional groups primarily found in a zwitter ionic resonance, where the nitrogen atoms are doubly linked to the neighbouring amide carbon atoms. Thus, the pyrimidinedione ring system's six atoms are all sp^2 hybridized and planar. As a result, caffeine's fused 5, 6 ring cores have ten pi electrons, making it aromatic by Hückel's rule.

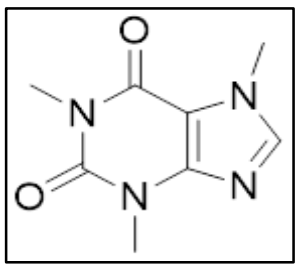

Fig 1: Chemical Structure of Caffeine

1.1 Synthesis of Caffeine

Caffeine may be synthesized in the lab, starting with dimethylurea and malonic acid.

1.2 Solubility

According to the IUPAC, solubility is the analytical makeup of a saturated solution represented as a proportion of a specific solute in a specific solvent. The solubility may be articulated using molality, molarity, mole ratio, mole fraction, mass (solute) per volume (solvent), and other concentration units. Solubility is the property of a chemical molecule defined as a solute to disperse in a solute-containing liquid. Essentially, the solvent, characteristic solvent properties, pressure, temperature, as well as pH of the solution are all factors that affect a substance's solubility. The saturation concentration occurs when more solute is introduced, and the concentration of the solution remains the same. Still, the excess solute begins to precipitate, revealing how much material is soluble in a certain solvent. A substance's solubility, or how easily it dissolves, differs greatly from its rate of solution or rate of dissolution. A substance's solubility should not be confused with its capacity to "dissolve," as the solution could also result from a chemical process. In hydrochloric acid, for instance, zinc "dissolves" (with effervescence) as a result of a chemical reaction that releases hydrogen gas in a displacement process. In the acid, the zinc ions are soluble[16].

1.3 Solubility Test

Caffeine's solubility is tested by dissolving the substance in a solution. Caffeine dosage forms are 100% soluble in chloroform and dissolved using various hydrophilic medicinal solutions. Ninety-five per cent methanol with water solubility.

1.4 Linearity and Range

The capacity of a technique to provide test findings that are inversely proportional to sample concentration throughout a specified range is known as linearity. In most cases, linearity is expressed as the regression line's slope variability. The range is the distance between the analyte's top and lower (inclusive) values that are quantified using the technique described precisely, accurately, and linearly. Normal expression of the range uses the same units as the test results achieved using the procedure. In addition to certain minimum stated ranges, the ICH standards stipulate a minimum of five concentration levels. Therefore, 80 to 120% of the target analyte is the lowest specified range for testing. According to ICH, the lowest suggested range must equal 80% of the concentration level. The range for impurity identification must also be greater than the quantization limit or between 50% and 1200% of each impurity's criteria. The samples were scanned in a UV-Vis Spectrophotometer against distilled water as various serial dilutions from the secondary stock solution (100 g/ml) ranging from 10 to 50 blanks were prepared. The chosen medication exhibited linearity between 10 to 50 g/ml ranges.

1.5 Robustness

According to the USP, toughness is measured by the degree of reproducibility of data acquired in various settings, such as various laboratories. The reproducibility of test results from lab to lab and analyst to analyst under typical, expected operational settings is measured by ruggedness. By analyzing aliquots from homogeneous lots in various laboratories, robustness is assessed. When analyzing the consistency of the findings when external elements like the analyst, laboratory, instrument, chemicals, and days are changed, robustness should be utilized as a metric to characterize the stability of the technique regarding alterations of the method's internal components. This includes factors like injection volume, column temperature, mobile phase composition, mobile phase rate of flow, and sample preparation. Another essential element of resilience is the procedure's capacity to remain consistent in sample matrix variability. An assessment of process robustness may aid in risk estimation and mitigation and even be used to encourage process development and future production. Instead of being tested into a product, robustness must be incorporated into the development and design process. Performance of the process and the product must be monitored throughout scale-up, introduction, and routine manufacture to guarantee robustness is maintained. The robustness of the approach was assessed by running the analysis at various max at 268 nm and 272 nm. The corresponding 5.0 µg/ml absorbencies were noted, and the result was displayed as a per cent RSD.

1.6 Relative Standard Deviation

The absolute value of the coefficient of variation is known as the relative standard deviation (RSD, sometimes known as per cent RSD). It's frequently stated as a percentage. It helps compare the degree of uncertainty among various measurements with different absolute magnitudes. An analytical procedure's robustness, which measures its ability to be unaffected by tiny but intentional changes in method parameters, gives a clue as to how reliable it will be under typical conditions. Robustness should ideally be investigated while the assay method is being developed. The most effective approach to accomplish this is by using a planned experiment. Examples of such experimental designs are a Plackett-Burman matrix strategy to study first-order effects or a 2k factorial design that will reveal information about the first (main) and higher-order (interaction) effects. To apply such a design, it is essential first to identify method variables that might be anticipated to impact the outcome. Think of an HPLC assay that makes use of an ion-pairing reagent. We might look at sample sonication or mixing time, column temperature, the makeup of the mobile phase organic solvent, the pH of the mobile phase, injection volume, flow rate, the concentration of the modifier, the ion-pairing reagent's concentration, etc. Through this kind of development study, the factors that have the biggest impacts on the outcomes can be identified in several studies. The final selected ranges will be confirmed reliable by the actual technique validation. The choice of manufacturing technology can be used to control process performance and variability. Processes with good design decrease the possibility of human error, increasing robustness. Identifying the crucial characteristics and process qualities, their parametric tolerances, and the best methods to regulate them during the creation of products and processes is important. The manufacturing science that underpins a product and process comprises critical process capability, quality attributes, quality system infrastructure, process parameters, and process control and manufacturing technologies.

1.7 The principles of process robustness are as follows.

(A) Critical Quality Attributes (CQAs)

The CQAs are the quantifiable characteristics that have been determined as being crucial to ensuring the envisioned efficacy, purity, and safety of a transitional or the ultimate product to meet the quality requirements.

(B) *Critical Process Parameters (CPPs)*

CPPs are a process input that substantially and immediately impacts a CQA when altered outside a predetermined range. Therefore, differentiating among elements that affect essential quality attributes is key, as are those that impact productivity, output, worker safety, or other business goals. In addition, most processes must provide a total product ranging from bulk to semi-finished or finished. Therefore, understanding the effects of raw materials, the control of production equipment, the level of automation, or the prescriptive technique required to ensure proper control is vital.

(C) *Proven Acceptable Range (PAR) and Normal Operating Range (NOR)*

The initially chosen tolerance parameters are changed or inveterate to account for the experimental data as the manufacturing science is developed. This becomes the parameter's Proven Acceptable Range, where within the PAR, an operational range is established depending on the parameter's Normal Operating Range. As the CPPs have already been found and specified, the process may be controlled within preset limitations for those CPPs in a reliable process. A process that neither exhibits low process variability nor effective process control is one that neither consistently performs in a limited NOR. The specified process controls, equipment, and capability all play a role in the capacity to operate in NOR.

(D) *Variability: Source and Control*

Calibration tolerances, human variables for non-automated processes, process equipment capabilities, raw materials, sampling variability, testing procedure variability, and environmental conditions inside the testing site are typical sources of variability.

(E) Setting Tolerance Limits

To give acceptable qualities, lower and upper tolerances around a midway within the PAR of a parameter ought to be created. The stated limits should be realistic and chosen to account for the predicted fluctuation of parameters while supporting the acceptance criteria for the quality attribute. Robust manufacturing procedures that consistently generate high-quality pharmaceuticals and allow for flexibility to support ongoing process improvement. The manufacturer gains from developing a system that promotes improved process understanding and results in process robustness over quality enhancements and cost savings. A well-defined product development endeavour aims to transmit a reliable process that can confidently show that, when used within predefined parameters, it consistently produces products that meet predetermined quality criteria[17-19].

1.8 U. V. Spectroscopy

UV-Vis or U.V./Vis is the abbreviation for ultraviolet-visible spectroscopy, which includes reflectance and absorption spectroscopy in the U.V. spectral region. In other words, it uses light from the visible spectrum, including surrounding light spectrums (near-UV including near-infrared [NIR]). The optical spectrum's absorbance or reflectance directly influences how a substance's colour is perceived. Atoms and molecules undergo electronic transitions in this region of the electromagnetic spectrum. Fluorescence spectroscopy studies excursions from the return to the ground state, while absorption explores the transformation from the ground state to the excited state, allowing absorption spectroscopy an auxiliary to fluorescence spectroscopy. To excite their electrons to higher anti-bonding chemical orbitals, molecules with -or non-bonding electrons (n-electrons) might absorb the energy from visible or ultraviolet light. It is easier for electrons to get excited the longer the wavelength of light it can absorb (i.e., the lower energy gap between the LUMO and the HOMO). Four distinct transition types are -*, n*, -*, and n*. The energy needed for different transitions follows the pattern -*>n-*>-*>n-*. Utilizing such equipment has the advantage of analyzing microscopic samples and the spectra of larger samples with excellent spatial resolution. As a result, they are employed in forensic laboratories to examine the colours of glass pieces, microscopic paint chips, and individual textile strands for the presence of dyes and pigments. They are also used to measure the vitrinite reflectance to determine the energy content of coal and petroleum source rocks, as well as in biological and materials science studies[15].

1.9 Determination of λmax

The wavelength of a wave, such as that of sound or light, is measured by measuring the distance between two identical troughs (low points) or peaks (high points) in the wave's repeating pattern. The ratio of incident to transmitted radiant power through a medium is described by the common logarithm, referred to as "absorption". Absorbance has no dimensions and does not represent the length, even though it approaches 0, as even the route length approaches 0. The physical act of absorbing light is known as absorption, but absorbance is used to measure or attenuate (transmitted radiant power) [20-22].

2. MATERIALS AND METHODS

2.1 Apparatus

All spectral measurements were performed using a Shimadzu double beam UV-visible spectrophotometer model 2202 with a bandwidth of 2 nm, precision of 0.5 nm, and two matched quartz cells with a 1cm path length.

2.2 Materials

A commercial dosage form for propyphenazone, Caffeine, and paracetamol is sold under the brand SARIDON by Nicholas Piramal India Limited. In this combination, propyphenazone and paracetamol provide pharmacological action as analgesics, while Caffeine acts as a stimulant.

2.3 Experimental Work

2.3.1 Preparation of Diluent

Slightly warm purified water is used as a diluent.

2.3.2 Preparation of Stock Solution

Dissolved 100 mg of Caffeine dosage forms in 100 mL of diluent for standard stock solutions of 1mg/mL concentration.

2.3.3 Preparation of Working Solution

The above standard stock solution took 10 ml and dissolved in 50 mL of diluents in a 100 mL volumetric flask, and the volume was made up to 100 mL with diluent. The final solution concentration is 0.1 mg/ml with diluent used as a blank, the sample was scanned using a UV-Vis Spectrophotometer in the 200-400nm range, and the wavelength matched the sample's maximum absorbance—which is at 268nm—was noted[22-26].

2.3.4 Preparation of Different standard solution

Taken 3 mL of the above solution and dissolved in a 10 mL volumetric flask containing 5 mL diluent. Volume made up of diluent up to 10 mL.

2.3.5 Preparation of Different standard solution-2

Taken 1.5 mL of the above solution and dissolved in a 10 mL volumetric flask containing 5 mL diluent. Volume made up with diluent up to 10 mL.

2.3.6 Preparation of Different standard solution-3

Taken 0.8 mL of the above solution and dissolved in a 10 mL volumetric flask containing 5 mL diluent. Volume made up of diluent up to 10 mL.

2.3.7 Preparation of Different standard solution-4

Taken 0.4 mL of the above solution and dissolved in a 10 mL volumetric flask containing 5 mL diluent. Volume made up of diluent up to 10 mL.

2.3.8 Preparation of Different standard solution-5

Taken 0.2 mL of the above solution and dissolved in a 10 mL volumetric flask containing 5 mL diluent. Volume made up of diluent up to 10 mL.

2.4 Precision

The new method's repeatability and intermediate precision were evaluated by analyzing replicate Q.C. standard samples of Caffeine at 6 µg/mL, 12 µg/mL, and 18 µg/mL. The results' percentage RSD values for intraday and interday precision were given about the absorption values on the third day. Based on % RSD, it was determined that the analytical method's precision was reliable.

2.5 Accuracy

Recovery experiments determined the method's accuracy. Recovery investigations were carried out at spiking levels of 80, 100, and 120% of the test concentration. The acceptance criterion for accuracy is a mean value which should be within ±15% of the actual value.

2.6 Limit of detection and limit of quantitation

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily exactly quantified. The limit of quantitation (LOQ) is the lowest amount of analyte in a sample, which can be quantitated with precision and accuracy. For each determination, the y-intercept was calculated, and the standard deviation (S.D.) of the y-intercept was computed. From these values, LOD and LOQ were calculated based on the response and slope (S) of the regression equation obtained from the linearity studies as follows:

$$LoD = 3.3\left(\frac{\sigma}{S}\right)$$

Where σ = the standard deviation of the intercept
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out from the standard deviation of *y*-intercepts of regression lines.

3. STATISTICAL ANALYSIS

To examine the linearity and range, accuracy, intra-day and inter-day precision, LOD and LOQ, Robustness, and Ruggedness, the mean and standard deviation (S.D.) for each parameter were calculated using Microsoft Excel 2007.

4. RESULTS AND DISCUSSION

The linearity studies were carried out by tracing various standard solution concentrations against the corresponding absorbencies. It was discovered that Caffeine behaves linearly at concentrations between 0.01 to 5.0 µg/mL. The calibration curve demonstrates that the correlation coefficient value, which was found to be 0.99, complies with Beer's law's limit for the concentration range. The suggested method was discovered to be accurate, straightforward, exact, durable, sensitive, and cost-efficient. Since the validation tests' findings were satisfactory, this method can be successfully used to estimate the amount of Caffeine in the tablet dosage form. All results are found per previous researchers' results (Table 1 to 3) (Figure 2 to 4) [22-26].

Table 1: Optical characteristics of Caffeine		
S. N.	**Characteristics**	**Value**
1	Beer Law Limit	0.01-5.0 µg/ml
2	Correlation coefficient	0.99
3	Regression equation	0.42+0.009
4	λ_{max}	268.5 nm

Table 2: Robustness			
At λ_{max} 268.5 nm			
S. N.	**Concentration** (µg/ml)	**Absorbance**	**Statistical analysis**
1	1.57	0.885	Mean value=0.519 (n=5)
2	0.80	0.710	
3	0.40	0.474	
4	0.20	0.330	
5	0.10	0.196	

Table 3: Ruggedness			
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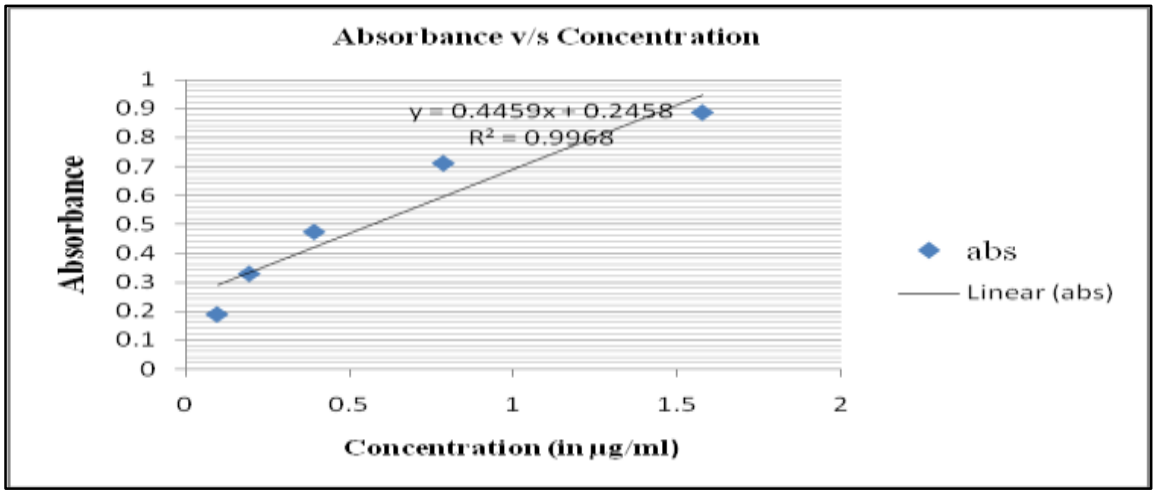

Fig 2: Linearity

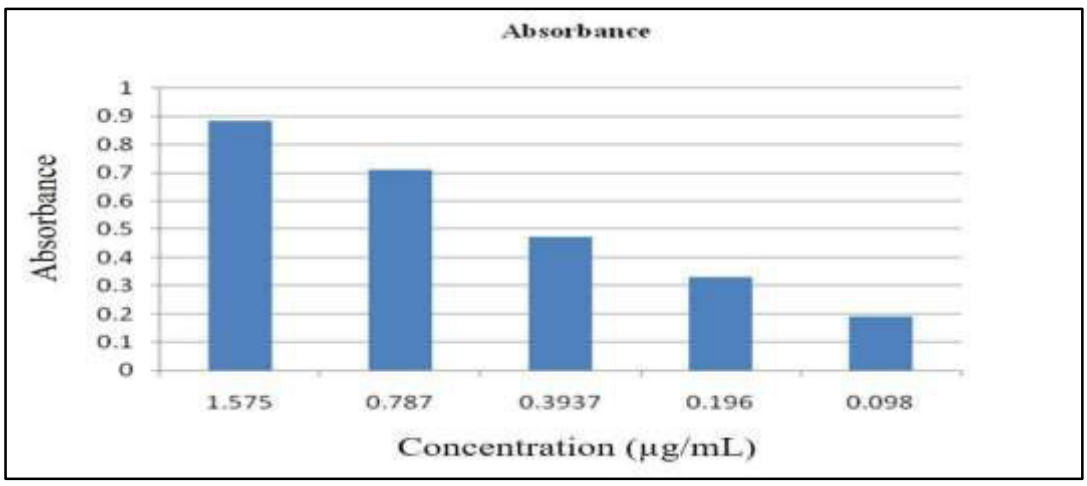

Fig 3: Robustness and Ruggedness

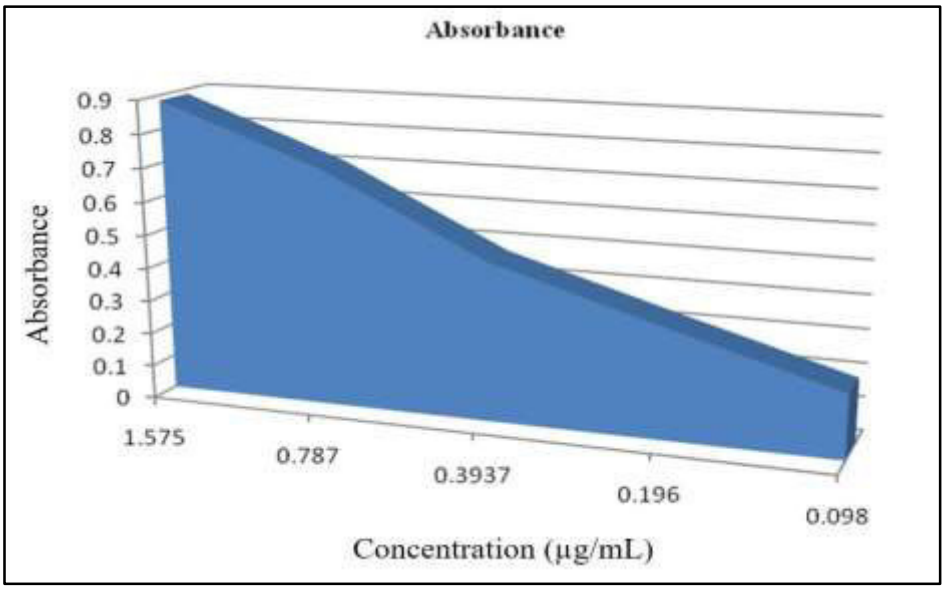

Fig 4: Spectrum Analysis

4.1 ***Precision***

The proposed method's accuracy was confirmed by re-analyzing the three distinct concentrations of Caffeine in a solution on the third day's intra-day and inter-day measurements. The results are expressed as a percentage of RSD (relative standard deviation) (Table 4). The accepted standard for precision is a percentage RSD of no more than 2%. The RSD (%) value for intra-day and inter-day observation was within 2%, which showed that the devised method for caffeine analysis had good precision and sensitivity.

Table 4: Precision studies of caffeine solution (λ_{max}= 268)

Intra-day Precision		Inter-day Precision	
Conc.	**RSD**	**Conc.**	**RSD**
6 µg/mL	0.304%	6 µg/mL	0.314%
12 µg/mL	0.421%	12 µg/mL	0.401%
18 µg/mL	1.123%	18 µg/mL	1.132%

4.2 ***Accuracy***

The developed methodologies undergo validation in accordance with ICH recommendations. The accuracy of the devised method was tested for the precise measurement of Caffeine in a solution using the standard addition method, and results were expressed as a percentage recovery. Table 5 displays the amount of drug recovered (µg/mL) and the % recovered at three different levels. The method's accuracy (% recovery) was discovered to be 100±15%, showing a reasonable agreement between the true and acquired data.

Table 5: Accuracy studies of caffeine solution (λ_{max}= 268)

Amount of Drug	Recovery Level (%)	Amount of drug added	Amount of drug found (n=3)	Recovery (%, n=3)
2 µg/mL	80	1.6 µg/mL	1.51 µg/mL	94.38±0.56
	100	2.0 µg/mL	2.11 µg/mL	105.50±1.06
	120	2.4 µg/mL	2.39 µg/mL	99.58±1.26

4.3 Limit of detection and limit of quantitation

The sensitivity of measurement of Caffeine by UV-visible spectrophotometric method was estimated in terms of limit of detection (LoD) and limit of quantification (LoQ). Obtained LoD and LoQ values indicate that the UV-spectrophotometric method can quantify drugs accurately in the microgram concentration range. LoD and LoQ for Caffeine were found to be 0.291 µg/mL and 0.912 µg/mL.

5. CONCLUSION

According to ICH recommendations, the parameters precision, linearity, specificity, ruggedness, robustness, and accuracy were investigated. For the drug validation, distilled water was utilized as a solvent, and the wavelength of 268.5 nm was chosen. Over a concentration range of 0.01 to 5.0µ g/ml, the medication followed Beer-rule. In addition, Lambert's Recovery studies evaluated the method's accuracy and found that it ranged from 99.46 to 100.67 per cent. Therefore, the technique was effectively used to regularly analyze this drug's formulation.

6. AUTHOR CONTRIBUTION STATEMENT

Ms Pooja Mittal and Dr Mukesh Kumar Kumawat designed the research work and performed laboratory work. Mr Sumit Tewatia and Dr Mukesh Kumar Kumawat prepared the original draft of the research paper. Dr Gufran Ajmal provided valuable inputs towards the design and corrections of the manuscript. All authors read and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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