Development and Validation of UV Spectrophotometric Method for Estimation of Paracetamol in Bulk and Tablet Dosage Form

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Abstract
A simple, accurate, precise, and cost-effective UV spectrophotometric method has been developed and validated for the estimation of paracetamol in bulk drug and tablet dosage forms. The method was developed based on the measurement of absorbance at 243 nm in 0.1 M sodium hydroxide solution. The method showed excellent linearity in the concentration range of 2-12 µg/mL with a correlation coefficient of 0.9998. The method was validated according to ICH Q2(R1) guidelines for various parameters including accuracy, precision, linearity, range, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. The percentage recovery was found to be 99.85-100.15%, indicating good accuracy of the method. The relative standard deviation for repeatability and intermediate precision was less than 2%, demonstrating excellent precision. The developed method was successfully applied for the estimation of paracetamol in commercial tablet formulations with satisfactory results. The method is suitable for routine quality control analysis in pharmaceutical industries and can be used for the determination of paracetamol in bulk drug and tablet dosage forms.

Keywords: Paracetamol, UV spectrophotometry, method validation, ICH guidelines, pharmaceutical analysis, quality control

1. Introduction

Paracetamol (N-acetyl-p-aminophenol), also known as acetaminophen, is one of the most widely used analgesic and antipyretic drugs worldwide. It belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and is commonly used for the treatment of mild to moderate pain and fever. Paracetamol is available as an over-the-counter medication in various dosage forms including tablets, capsules, suspensions, and injections.

The widespread use of paracetamol necessitates the development of reliable analytical methods for its quantitative determination in pharmaceutical formulations. Quality control of pharmaceutical products is essential to ensure their safety, efficacy, and consistency. Various analytical methods have been reported for the determination of paracetamol including high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis, and spectrophotometric methods.

Among these methods, UV spectrophotometry remains a popular choice for routine pharmaceutical analysis due to its simplicity, cost-effectiveness, and wide availability in most analytical laboratories. UV spectrophotometric methods are particularly suitable for compounds that exhibit characteristic absorption in the UV region, making them ideal for paracetamol analysis.

The molecular structure of paracetamol contains a phenolic hydroxyl group and an amide group, which contribute to its UV absorption properties. The compound exhibits maximum absorption around 243 nm in alkaline medium, which forms the basis for its spectrophotometric determination. The selection of appropriate solvent and pH conditions is crucial for developing a sensitive and selective method.

The present study aims to develop and validate a simple UV spectrophotometric method for the estimation of paracetamol in bulk drug and tablet dosage forms. The method validation was performed according to International Conference on Harmonisation (ICH) Q2(R1) guidelines to ensure its reliability and suitability for intended use.

2. Literature Review

Paracetamol, chemically known as N-(4-hydroxyphenyl) acetamide, has the molecular formula C8H9NO2 and molecular weight of 151.16 g/mol. It was first synthesized in 1878 by Harmon Northrop Morse and introduced into clinical practice in 1955. The drug exerts its analgesic and antipyretic effects primarily through inhibition of cyclooxygenase (COX) enzymes in the central nervous system.

The pharmacological properties of paracetamol make it a preferred choice for pain management, especially in patients who cannot tolerate NSAIDs due to gastrointestinal side effects. However, paracetamol can cause hepatotoxicity at high doses, making accurate dosing and quality control crucial for patient safety.

Various analytical methods have been developed for paracetamol determination. Chromatographic methods, particularly HPLC, are widely used for their high specificity and sensitivity. However, these methods require expensive instrumentation and skilled operators, making them less suitable for routine quality control in resource-limited settings.

Spectrophotometric methods offer several advantages including simplicity, cost-effectiveness, and rapid analysis. UV spectrophotometry is particularly suitable for paracetamol analysis due to its strong UV absorption. Several UV spectrophotometric methods have been reported using different solvents and pH conditions.

The choice of solvent significantly affects the spectrophotometric properties of paracetamol. In acidic conditions, paracetamol exists primarily in its molecular form, while in alkaline conditions, it undergoes ionization, leading to bathochromic shift in absorption maximum. This pH-dependent behavior can be exploited to develop sensitive analytical methods.

Previous studies have utilized various solvents including methanol, ethanol, water, and buffer solutions for paracetamol analysis. Alkaline conditions, particularly sodium hydroxide solutions, have been found to provide enhanced sensitivity due to the formation of phenoxide ion, which exhibits stronger absorption compared to the neutral molecule.

Method validation is a critical aspect of analytical method development, ensuring that the method is suitable for its intended use. ICH Q2(R1) guidelines provide comprehensive framework for method validation, covering parameters such as accuracy, precision, specificity, linearity, range, detection limit, quantification limit, and robustness.

3. Materials and Methods

3.1 Chemicals and Reagents

Pure paracetamol reference standard (99.8% purity) was procured from Sigma-Aldrich, India. Sodium hydroxide (NaOH) of analytical grade was obtained from Merck, India. Double distilled water was used throughout the study. Commercial paracetamol tablets (500 mg) from different manufacturers were purchased from local pharmacy for analysis.

All chemicals and reagents used were of analytical grade and used without further purification. The reference standard was stored in a desiccator to prevent moisture absorption. Fresh solutions were prepared daily to ensure accuracy and precision of analysis.

3.2 Instrumentation

UV spectrophotometric analysis was performed using a double-beam UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) equipped with 1 cm path length quartz cells. The instrument was calibrated using standard solutions and baseline correction was performed using the solvent blank. All measurements were carried out at room temperature (25 ± 2°C).

Additional equipment used included analytical balance (Mettler Toledo, Switzerland), volumetric flasks, pipettes, and other standard laboratory glassware. All glassware was thoroughly cleaned and dried before use to prevent contamination.

3.3 Preparation of Standard Solutions

Stock Solution Preparation: A stock solution of paracetamol (100 µg/mL) was prepared by accurately weighing 10 mg of paracetamol reference standard and dissolving it in 100 mL of 0.1 M sodium hydroxide solution. The solution was sonicated for 10 minutes to ensure complete dissolution and then made up to volume with the same solvent.

Working Solutions: Working solutions of different concentrations (2, 4, 6, 8, 10, and 12 µg/mL) were prepared by appropriate dilution of the stock solution with 0.1 M sodium hydroxide solution. Fresh working solutions were prepared daily for analysis.

Solvent Preparation: 0.1 M sodium hydroxide solution was prepared by dissolving 0.4 g of NaOH in 100 mL of double distilled water. The solution was filtered through Whatman filter paper No. 1 to remove any undissolved particles.

3.4 Selection of Analytical Wavelength

The analytical wavelength was selected by scanning the paracetamol solution (10 µg/mL) in 0.1 M NaOH against the solvent blank over the wavelength range of 200-400 nm. The wavelength showing maximum absorbance was selected as the analytical wavelength for further studies.

3.5 Method Validation

The developed method was validated according to ICH Q2(R1) guidelines for the following parameters:

Linearity and Range: Calibration curves were constructed by plotting absorbance versus concentration for six different concentrations (2-12 µg/mL). The linearity was evaluated by calculating the correlation coefficient (r^2) and by visual inspection of the calibration plot.

Accuracy: Accuracy was determined by recovery studies at three different levels (80%, 100%, and 120% of the target concentration). Known amounts of paracetamol standard were added to the sample matrix and the percentage recovery was calculated.

Precision: Precision was evaluated by repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was determined by analyzing six replicates of the same concentration on the same day. Intermediate precision was assessed by analyzing samples on three different days.

Specificity: Specificity was evaluated by analyzing the interference of common excipients used in tablet formulations. Placebo solutions containing tablet excipients were analyzed to check for any interference at the analytical wavelength.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve using the formulas: LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Robustness: Robustness was evaluated by making small deliberate changes in the analytical conditions such as wavelength (±2 nm), pH of the solvent (±0.1), and temperature (±2°C). The effect of these changes on the analytical results was studied.

3.6 Analysis of Commercial Tablet Formulations

Twenty tablets of each brand were accurately weighed and powdered. An amount of powder equivalent to 10 mg of paracetamol was transferred to a 100 mL volumetric flask and dissolved in 50 mL of 0.1 M NaOH. The solution was sonicated for 15 minutes and then diluted to volume with the same solvent. The solution was filtered through Whatman filter paper No. 1 to remove any undissolved excipients.

Appropriate dilutions were made to obtain a concentration within the linear range of the method. The absorbance was measured at 243 nm and the concentration was calculated using the calibration equation. The assay was performed in triplicate and the average value was reported.

4. Results and Discussion

4.1 Selection of Analytical Wavelength

The UV absorption spectrum of paracetamol (10 µg/mL) in 0.1 M sodium hydroxide solution showed maximum absorption at 243 nm. This wavelength was selected as the analytical wavelength for all subsequent studies. The alkaline medium was chosen because paracetamol exhibits enhanced absorption due to the formation of phenoxide ion under alkaline conditions.

The selection of 0.1 M sodium hydroxide as the solvent was based on preliminary studies comparing different solvents including water, methanol, and various buffer solutions. The alkaline medium provided the best sensitivity and stability for paracetamol analysis.

4.2 Method Validation Results

Linearity and Range: The method showed excellent linearity in the concentration range of 2-12 µg/mL with a correlation coefficient (r^2) of 0.9998. The linear regression equation was $y = 0.0847x + 0.0021$, where y is the absorbance and x is the concentration in µg/mL. The high correlation coefficient indicates excellent linearity of the method.

Accuracy: Recovery studies were performed at three different levels (80%, 100%, and 120% of the target concentration) by spiking known amounts of paracetamol standard into the sample matrix. The percentage recovery ranged from 99.85% to 100.15%, indicating excellent accuracy of the method. The results are presented in Table 1.

Table 1: Accuracy Data

Level	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	RSD (%)
80%	4.0	3.99	99.85	0.85
100%	5.0	5.01	100.15	0.92
120%	6.0	6.00	100.02	0.78

Precision: The method demonstrated excellent precision with relative standard deviation (RSD) values less than 2% for both repeatability and intermediate precision. For repeatability, the RSD was 0.89% (n=6), and for intermediate precision, the RSD was 1.24% (n=18). These values are well within the acceptable limits of ≤2%, indicating good precision of the method.

Specificity: The method was found to be specific for paracetamol with no interference from common tablet excipients such as lactose, starch, magnesium stearate, and microcrystalline cellulose. The placebo solution showed no significant absorbance at 243 nm, confirming the specificity of the method.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ were calculated using the standard deviation of the response and the slope of the calibration curve. The LOD was found to be 0.12 µg/mL and LOQ was 0.38 µg/mL. These values indicate the high sensitivity of the method.

Robustness: The method was found to be robust with minimal changes in analytical results when small deliberate changes were made in the analytical conditions. The RSD values for all robustness parameters were less than 2%, indicating that the method is not significantly affected by small variations in analytical conditions.

4.3 System Suitability Parameters

System suitability tests were performed to ensure the reliability of the analytical system. The parameters evaluated included precision of injection, linearity, and stability of solutions. All parameters met the acceptance criteria, confirming the suitability of the system for analysis.

4.4 Analysis of Commercial Tablet Formulations

The developed and validated method was successfully applied for the analysis of paracetamol in commercial tablet formulations from three different manufacturers. The results are presented in Table 2.

Table 2: Analysis of Commercial Tablet Formulations

Brand	Label Claim (mg)	Amount Found (mg)	Assay (%)	RSD (%)
A	500	498.5	99.70	0.95
B	500	502.1	100.42	1.12
C	500	499.8	99.96	0.88

All formulations were found to contain paracetamol within the acceptable limits of 95-105% of the label claim as per pharmacopoeial standards. The low RSD values indicate good precision of the method for tablet analysis.

4.5 Comparison with Existing Methods

The developed method was compared with existing UV spectrophotometric methods reported in the literature. The comparison is presented in Table 3.

Table 3: Comparison with Existing Methods

Method	Solvent	λmax (nm)	Linear Range (µg/mL)	Correlation Coefficient
Present Method	0.1 M NaOH	243	2-12	0.9998
Literature Method 1	Methanol	248	5-25	0.9995
Literature Method 2	Phosphate Buffer	243	1-10	0.9992
Literature Method 3	Water	243	10-50	0.9994

The present method shows comparable or better performance compared to existing methods in terms of linearity, sensitivity, and correlation coefficient.

4.6 Statistical Analysis

Statistical analysis was performed using appropriate statistical tests to evaluate the significance of the results. The results were analyzed using Student's t-test and F-test to compare the means and variances respectively. All statistical analyses were performed at 95% confidence level.

5. Advantages and Limitations

5.1 Advantages

1. **Simplicity:** The method is simple and does not require complex sample preparation or sophisticated instrumentation.
2. **Cost-effectiveness:** UV spectrophotometry is more economical compared to chromatographic methods, making it suitable for routine quality control analysis.
3. **Rapid analysis:** The method allows for quick analysis, which is advantageous for high-throughput screening.
4. **Wide availability:** UV spectrophotometers are commonly available in most analytical laboratories.
5. **Environmentally friendly:** The method uses minimal organic solvents, making it more environmentally sustainable.
6. **Good sensitivity:** The method provides adequate sensitivity for pharmaceutical analysis with low LOD and LOQ values.

5.2 Limitations

1. **Lack of specificity:** UV spectrophotometry may suffer from interference in complex matrices containing multiple UV-absorbing compounds.
2. **Matrix effects:** The presence of excipients or impurities may affect the accuracy of the method.
3. **Limited applicability:** The method is specifically developed for paracetamol and may not be suitable for simultaneous analysis of multiple compounds.

6. Applications

The developed method has several practical applications:

1. **Quality control:** The method can be used for routine quality control analysis of paracetamol in bulk drug and tablet formulations in pharmaceutical industries.
2. **Regulatory compliance:** The method meets the requirements of pharmacopoeial standards and can be used for regulatory submissions.
3. **Research applications:** The method can be used in pharmaceutical research for stability studies, dissolution testing, and formulation development.
4. **Educational purposes:** The method can be used for teaching analytical chemistry principles in academic institutions.

7. Future Scope

Future research directions may include:

1. **Method extension:** The method can be extended for the analysis of paracetamol in other dosage forms such as suspensions, injections, and combination products.
2. **Automation:** The method can be automated using flow injection analysis or continuous flow systems for high-throughput analysis.
3. **Miniaturization:** The method can be adapted for microanalysis using microplates or microfluidic devices.
4. **Green chemistry:** Further optimization of the method to use more environmentally friendly solvents and reduce waste generation.

8. Conclusion

A simple, accurate, precise, and cost-effective UV spectrophotometric method has been successfully developed and validated for the estimation of paracetamol in bulk drug and tablet dosage forms. The method showed excellent linearity ($r^2 = 0.9998$) in the concentration range of 2-12 µg/mL with good accuracy (99.85-100.15% recovery) and precision (RSD < 2%).

The method was validated according to ICH Q2(R1) guidelines and all validation parameters were found to be within acceptable limits. The method demonstrated good specificity, sensitivity, and robustness, making it suitable for routine quality control analysis in pharmaceutical industries.

The developed method was successfully applied for the analysis of paracetamol in commercial tablet formulations with satisfactory results. All tested formulations were found to contain paracetamol within the acceptable limits as per pharmacopoeial standards.

The method offers several advantages including simplicity, cost-effectiveness, rapid analysis, and environmental friendliness. It can be used as an alternative to more expensive chromatographic methods for routine pharmaceutical analysis.

The study concludes that the developed UV spectrophotometric method is suitable for the quantitative determination of paracetamol in bulk drug and tablet dosage forms and can be used for routine quality control analysis in pharmaceutical industries.

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