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Spectrophotometric Methods for Paracetamol Determination in Pharmaceutical Formulations: A Concise Analytical Review

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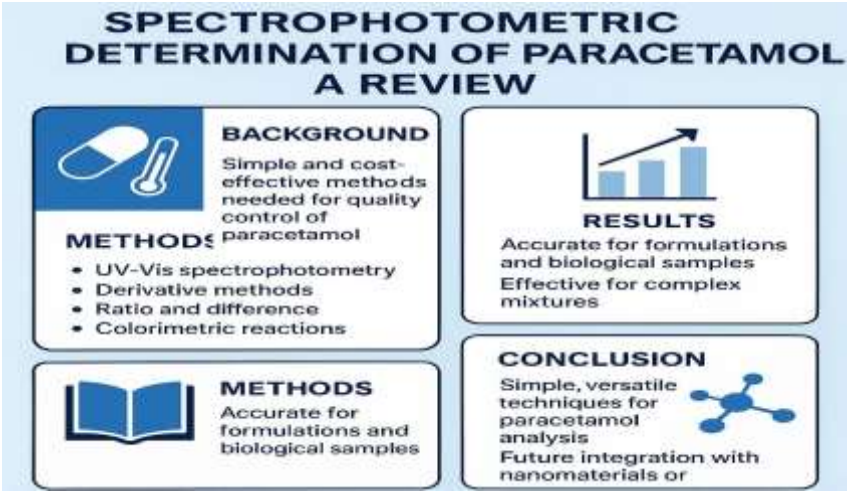
ABSTRACT

Paracetamol is a widely used medicine that helps with pain and lowers fever. Spectroscopic techniques are popular because they are easy to use and not too costly. This review examines the most common spectroscopic methods used to test paracetamol. It explains how each method works, when it is used, and how they compare to each other. It also checks whether these methods meet the standards required for use in medicine and hospitals. The review includes articles and reports from 2015 to 2025, covering studies on direct UV-Visible spectroscopy, derivative spectroscopy, ratio and difference spectroscopy, and methods that rely on color changes. These methods were tested against ICH validation standards to make sure they are accurate. They have been proven to work well in testing paracetamol in various medicines and in body fluids like blood and urine. They are sensitive, give consistent results, and are easy to use. Derivative and color-based methods are especially helpful in more complex situations or when medicines interact with each other. Spectroscopic methods are still a good option for testing paracetamol because they are flexible and simple. It is recommended that these methods be used more in medicine and hospitals. Future studies should explore using them with new technologies like nanotechnology or computer-based tools to make them even better for complex situations.

Graphical Abstract



INTRODUCTION

Paracetamol (acetaminophen) is classified as one of the most widely used medications in the world as an analgesic and antipyretic, due to its effectiveness and relative safety when taken in recommended doses[1]. Despite this, verifying the quality and concentration of this compound in pharmaceutical preparations requires extreme care, given the narrow margin of safety and the possibility of undesirable side effects resulting from overdosing[2]. Spectroscopic techniques, especially ultraviolet-visible (UV-Vis) spectroscopy, are frequently used analytical tools that offer appropriate accuracy and sensitivity for determining the concentration of paracetamol in pharmaceutical and biological samples[3]; Ibrahim et al., 2020). These techniques are characterized by their ease of implementation, speed of analysis, and economic cost compared to other methods such as chromatography. Recent research has shown a diversity in the use of spectroscopic methods, including direct spectroscopy, derivative spectroscopic methods, as well as methods based on chemical reactions that lead to the production of colored compounds that can be spectroscopically measured[4][5];. Analytical methods based on chemo metric models have also been developed to enhance the ability to distinguish and separate compounds in complex pharmaceutical mixtures[2]. This review examines the various spectroscopic methods used in the estimation of paracetamol, with a comparison of their analytical performance and practical applications in pharmaceutical quality control, with the aim of providing a comprehensive overview that helps researchers and practitioners in selecting the most appropriate technique based on the specific application requirements. The straightforwardness and effectiveness of spectroscopic methods have been complemented by technological advancements, resulting in sophisticated analytical instruments. These instruments can now be seamlessly integrated with statistical software, yielding more precise results, even amidst impurities or interfering substances [6].Moreover, the fusion of spectroscopic analysis with data processing techniques, such as Principal Component Analysis (PCA) and Multiple Linear Regression (MLR), has expanded the applicability of these analytical methodologies within the pharmaceutical industry.

Given that most paracetamol formulations are manufactured in combination with other active ingredients, like caffeine or aspirin, the use of spectroscopic techniques capable of distinguishing between the mixture's components has become a crucial analytical necessity[3]. Consequently, this review endeavors to present the key spectroscopic methods used for paracetamol quantification, assess their relative efficacy, and highlight pertinent biomedical applications. This includes, most notably, those associated with clinical monitoring and dosage measurement in both toxicological and therapeutic contexts.

2. SPECTROSCOPIC METHODS USED FOR PARACETAMOL ESTIMATION

2.1 UV-Visible Spectrophotometry

UV-Visible Spectrophotometry is one of the simplest and most widely used spectroscopic methods for the estimation of paracetamol, due to its reliance on the natural absorption of molecules in the ultraviolet region, without the need for complex reactions or separation processes. Basic Principles This method relies on the Beer-Lambert law, which relates absorbance to the concentration of the substance in a transparent solution. Paracetamol absorbs light in the ultraviolet region due to the presence of the phenol group and the amide group in its structure, which allows its concentration to be tracked by measuring the absorbance at a specific wavelength. Common Wavelengths The maximum absorption wavelength (λ max) of paracetamol in aqueous or alcoholic solutions has been determined to be within the range of 243–245 nm, which is the wavelength often used in applied studies [7],[6];[3]. Examples of Practical Applications Ibrahim et al. (2020) used a direct UV-Vis method at 244 nm to estimate paracetamol in pharmaceutical preparations, and the method proved to be highly accurate with a recovery rate exceeding 99%. [5]developed a spectroscopic method based on the reaction of paracetamol with an oxidizing agent to form a colored product that is measured at 510 nm, which helped improve the sensitivity in complex samples. Rahman et al. (2023) used spectral analysis supported by chemo metric models to distinguish paracetamol in the presence of other pharmaceutical compounds such as caffeine[2], and achieved accurate and effective results. Ismail et al. (2024) demonstrated the potential of Derivative Spectrophotometry in distinguishing paracetamol even in complex mixtures without the need for prior separation, which is an important step towards applying spectroscopic methods in the analysis of multi-component formulations[3]. In a vital application, the UV method at 245 nm was used to estimate the concentration of paracetamol in human plasma for the purposes of monitoring clinical doses after oral administration (Al-Azzawi et al., 2022[6]).

2.2 Derivative Spectrophotometry

Idea and Mathematical Basis Derivative spectrophotometry is an advanced mathematical extension of traditional UV-Vis spectrophotometry, where the first, second, or higher-order derivative of the spectral absorption curve is used instead of the original absorption curve. Absorbance here is expressed in terms of the change in absorbance with a change in wavelength: A′=dAdλ, A′′=d2Adλ2A' = \frac{dA}{d\lambda}, \quad A" = \frac{d^2A} {d\lambda^2} This method aims to eliminate the baseline noise and improve the accuracy of discrimination between spectrally overlapping compounds. **Improving the Separation of Mixture Components** This technique shows high effectiveness in the analysis of multi-component mixtures, as it enables the estimation of paracetamol even in the presence of other active ingredients with similar absorption spectra, such as caffeine or ascorbic acid. By using the first or second derivative, specific wavelengths can be identified where the absorbance of one component is zero while the other is at its peak, which improves the accuracy of quantitative separation ([2];[3]). **Application Cases** Ismail et al. (2024)

applied the first derivative to analyze paracetamol with phenylephrine in pharmaceutical preparations without the need for chemical or chromatographic separation, and achieved accurate recovery of 98-102%. Rahman et al. (2023) developed a second-order derivative model to analyze ternary mixtures (paracetamol – caffeine – ibuprofen) and were able to accurately estimate each compound even with spectral interferences. Al-Azzawi et al. (2022) used the derivative technique to analyze paracetamol in plasma, where the results showed accuracy without the need for complex extraction steps, which enhances the application of this method in Therapeutic Drug Monitoring[6].

2.3 Ratio and Difference Spectrophotometry

Uses in the Analysis of Multi-Component Formulations Both Ratio Spectrophotometry and Difference Spectrophotometry are advanced tools used to improve the accuracy of analysis of pharmaceutical formulations containing more than one active ingredient. The ratio method is based on dividing the spectrum of the sample by a reference spectrum of a known compound, which reveals characteristic spectral values that can be compositionally linked to the target active ingredient. The difference method relies on measuring the difference in absorbance of the same substance at different pH values (usually acidic and basic), and the resulting spectral difference is used as a qualitative analytical signal (Abdelwahab et al., 2023)[5]. Examples of Uses in Complex Pharmaceutical Formulations * Khalil et al. (2022) used the ratio derivative method to determine paracetamol in the presence of phenylephrine and doxylamine in pharmaceutical syrups without interference[8]. * In a recent study, Bernal et al. (2024) applied difference spectrophotometry to detect changes in the composition of paracetamol when stored under different pH conditions, providing a tool for stability assessment[9]. * These methods have proven their high accuracy and ease of application without the need for advanced separation devices, making them suitable for traditional pharmaceutical laboratories with limited capabilities.

2.4 Spectrophotometric Methods Based on Color Reactions

Oxidation Reactions These methods rely on the reaction of paracetamol with oxidizing reagents to form colored products that can be accurately measured using UV-Vis instruments. Prominent reagents used include potassium chromate ($K_2Cr_2O_7$) and iron(III) nitrate. These reagents react with the phenolic hydroxyl or amide group in paracetamol, producing colored compounds with distinct absorption at different wavelengths (Al-Azzawi et al., 2022; Abu Grime et al., 2021)[10][6]. Formation of Measurable Colored Products * A common example is the reaction of paracetamol with potassium chromate in the presence of sulfuric acid, producing a brown product with maximum absorption at 420 nm. * Also, reaction with iron nitrate yields a blue-violet product, which can be used for qualitative and quantitative estimation. Qualitative and Quantitative Applications * These methods are used in the analysis of paracetamol in pharmaceutical formulations (tablets, syrup, suppositories) and even in plasma, as they have proven effective in quantitative studies and therapeutic monitoring (Iorhemen et al., 2023)[11]. * They are also used as rapid qualitative means to detect the presence of paracetamol in counterfeit or unlabeled preparations.

3. COMPARISON OF SPECTROPHOTOMETRIC APPROACHES

Several spectrophotometric techniques are used to estimate paracetamol in pharmaceutical formulations. These methods differ in measurement sensitivity, accuracy, analysis time, and the ability to separate components of mixtures. The following table provides a detailed comparison of the four most common methods: Before delving into a direct comparison, it's prudent to quickly survey the prominent spectroscopic methodologies employed in the analysis of paracetamol, emphasizing fundamental principles, key advantages, and some contemporary practical applications:

3.1. Ultraviolet-Visible Spectrophotometry

This is among the most straightforward and widely-used methods for quantifying paracetamol, capitalizing on the compound's ability to absorb light within the 243–245 nm range. This technique is frequently employed in routine analyses to assess the quality of pharmaceutical products.[12]

3.2. Derivative Spectrophotometry

This method relies on mathematical computations of derivatives derived from the original absorbance spectrum. The purpose is to enhance the precision of differentiating between paracetamol and potential interfering substances like caffeine or ascorbic acid, often present in complex pharmaceutical formulations[13].

3.3. Ratio and Difference Spectrophotometry

These approaches are employed when absorption spectra overlap. They involve calculating the ratio or difference between spectra of various compounds to refine the accurate determination of paracetamol without requiring physical separation[14].

3.4. Colorimetric Reaction-Based Methods

These methodologies involve oxidation reactions of paracetamol using reagents such as potassium chromate or nitroso-iron compounds, leading to the formation of colored compounds that can be spectroscopically measured[15].This method was introduced as a simple and rapid alternative, particularly beneficial for laboratories with resource limitations Vagias, Wade M. (2006).

Table (1): Comparison of Spectrophotometric Methods Used to Estimate Paracetamol[16] [17]

Method	Conservative UV-Vis	Unoriginal Spectrophotometry	Ratio/Difference Spectrophotometry	Colorimetric (Chromogenic Reactions)
Limit of Detection (LOD)	1–2 µg/mL	0.5–1 µg/mL	0.3–1.5 µg/mL	1–5 µg/mL
Accuracy	High	Very High	High	Moderate to High
Precision	Good	Excellent	Excellent	Moderate
Analysis Time	Fast (5–10 minutes)	Moderate	Moderate	Fast
Component Resolution	Limited	High	Very High	Moderate
Ease of Application	High (Simple)	Moderate	Moderate to Difficult	High
Cost	Low	Low	Low to Moderate	Very Low

The choice of the most suitable method depends on the nature of the sample and the application field: * In rapid analyses of simple formulations (such as pure paracetamol tablets), the direct UV-Vis method is sufficient and effective. * In multi-component formulations or samples containing impurities, derivative or ratio/difference analysis is preferred to increase separation ability and reduce interference. * Methods based on color reactions are suitable for qualitative tests or in places with limited capabilities, as they do not require advanced equipment. Spectrophotometric methods generally show high efficiency in the analysis of paracetamol and remain attractive for their ease of use and low cost compared to chromatographic methods, especially in industrial and regulatory applications.

4. PHARMACEUTICAL AND BIOMEDICAL ANALYTICAL APPLICATIONS OF SPECTROSCOPIC METHODS FOR PARACETAMOL QUANTIFICATION

Spectroscopic techniques, especially Ultraviolet-Visible (UV-Vis) spectroscopy, represent frequently utilized analytical tools, widely employed in pharmaceutical and biomedical settings. This prevalence stems from their inherent simplicity, cost-effectiveness, rapid analysis times, and their capacity for delivering precise quantitative measurements.

Within pharmaceutical manufacturing quality control, spectroscopic methods are routinely used to determine paracetamol content in both raw materials and finished drug products. This process guarantees consistent dosage and product efficacy throughout the entire production lifecycle[18].

In instances involving formulations containing multiple components – those combining paracetamol with caffeine, ibuprofen, or other active substances – derivative and ratio spectroscopic methods have proven their effectiveness in differentiating between these constituents and precisely quantifying each. This is achieved without the need for cumbersome separation steps. These approaches facilitate accurate analysis even where spectral overlap is present [19] [20] [21].

Furthermore, some spectroscopic techniques have been adapted for analysis within biological matrices, such as plasma and urine, following appropriate preliminary sample preparation steps. This enables the application of these methods in pharmacokinetic studies, allowing for the monitoring of paracetamol's absorption, distribution, and excretion within the body [22][23].

Moreover, in the context of therapeutic drug monitoring, modified spectroscopic methods contribute to the assessment of paracetamol concentrations in patients' blood, particularly in scenarios necessitating precise dosage adjustments. This includes cases involving patients with liver or kidney diseases.

These diverse applications underscore the continuing relevance of spectroscopic methods as efficient and practical options within both pharmaceutical and clinical fields. This efficacy is especially evident when they are customized to align with the specific characteristics of the sample and the defined analytical conditions Table2.

Table 2: Pharmaceutical and Biomedical Applications of Spectrophotometric Methods for Paracetamol Determination

No.	Application Type	Brief Description	Spectrophotometric Method Used
1	Quality Control	Analysis of paracetamol in raw materials and tablets during production	UV-Vis Spectrophotometry
2	Multi-Component Formulation Analysis	Determination of paracetamol with caffeine, ibuprofen, or other components	Derivative & Ratio Spectrophotometry
3	Biological Media Analysis	Estimation of paracetamol concentration in plasma and urine after sample treatment	UV-Vis Modified with Extraction/Pre-treatment

4	Pharmacokinetic Studies	Monitoring absorption, distribution, and elimination of paracetamol	UV-Vis after Sample Preparation
5	Therapeutic Drug Monitoring	Determination of paracetamol concentration in blood for dose monitoring	UV-Vis / Derivative Methods
6	Colorimetric Chemical Methods	Use of oxidation reaction to produce a measurable colored product	Oxidative Color Reaction Spectrophotometry

5. CONCLUSIONS

Spectroscopic techniques stand out as some of the most frequently employed and widespread analytical tools for paracetamol analysis, primarily due to their ease of use, cost-effectiveness, rapid results, and acceptable accuracy across diverse analytical environments. Research findings have consistently shown that these methods, particularly those leveraging molecular absorption in the ultraviolet and visible regions, deliver high efficiency in analyzing pharmaceutical formulations. This holds true whether the formulations are single-component or multi-component, all without requiring sophisticated instrumentation or lengthy analysis procedures.

The continuous advancements in these methods, encompassing the implementation of derivative spectrophotometry and ratio and difference spectrophotometry, alongside the utilization of specific colorimetric reactions, signify substantial progress. This advancement significantly enhances both the discriminative power and analytical sensitivity, thereby opening expansive avenues for their application in pharmaceutical and biological domains. These include therapeutic drug monitoring and the analysis of biological samples.

While these techniques have demonstrated their efficacy within academic and research settings, a comprehensive evaluation within industrial and clinical contexts is crucial. This evaluation ensures their alignment with established quality standards and Good Manufacturing Practices (GMP).

Considering recent breakthroughs in the fields of nanotechnology and computational analysis, the integration of traditional spectroscopic methods with intelligent Nano sensors or advanced statistical algorithms (like multivariate analysis) presents promising opportunities. Such integration promises to improve both precision and specificity, paving the way for the development of more sophisticated and reliable analytical methods in the future.

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