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Method Validation for Determination of Paracetamol in Tablet Dosage Form Using UV-Visible Spectrophotometer

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A B S T R A C T

A simple, accurate, and cost-effective UV-spectrophotometric method for determining Paracetamol in tablet pharmaceutical formulations has been developed. The method was designed and validated in accordance with the criteria of the International Conference on Harmonization (ICH). The developed method was validated statistically concerning linearity range, precision, accuracy, the limit of detection and the limit of quantitation. The study used ethanol-water as a solvent, and λ_{max} was found to be 243 nm. In the range of 2.0–16.0 µg mL^{-1}, a pure drug concentration was prepared, and linear regression analysis revealed a good linear connection with an R^2 value of 0.999. The detection and quantification limits were determined to be 0.5 and 1.5 µg mL^{-1}, respectively. The recoveries ranged between 103 and 109%, and the relative standard deviation was less than 2%, indicating that this method was developed for the potential determination of Paracetamol in tablet dosage formulations.

1.0 Inroduction

Paracetamol (PC), often known as acetaminophen or 4-acetamidophenol, is a common painkiller and fever-reduction medicine and one of the most frequently used analgesic drugs. Nacetyl-p-aminophenol is its scientific name, and its chemical formula is C8H8N2O2 (Fig. 1). Harmon Northrop Morse, an American scientist, first synthesized paracetamol in 1878 (Charpiat et al., 2013). It is a generic version of a drug sold under various brand names, such as Tylenol and Panadol (Sridevi et al., 2020). It is a common component of many over-the-counter cold and flu remedies that are offered commercially and used to treat various symptoms (Hinz et al., 2008). It is administered orally or rectally most of the time. However, it is also available for intravenous use. The various forms of paracetamol include tablets, pills, capsules, injections, and syrup (Santos et al., 2020).

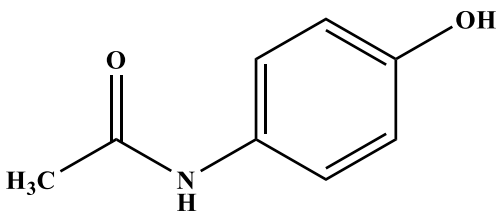

Fig. 1. Chemical structure of Paracetamol (PC).

According to some publications, it is categorized as a non-steroidal anti-inflammatory medicine (NSAID); however, other sources do not consider it an NSAID. The vast majority of sources make this distinction between them clear, for example, by listing NSAIDs and paracetamol in the same line. In comparison to NSAIDs, paracetamol does not have nearly as many anti-inflammatory properties (Andersson et al., 2011). On the other hand, aspirin, paracetamol, and other non-steroidal anti-inflammatory drugs (NSAIDs) show varying levels of analgesic, anti-inflammatory, antipyretic, and antiplatelet actions. This is because they all act by the exact mechanism, which is the inhibition of prostaglandin synthesis by inhibiting cyclooxygenase (COX). To date, paracetamol's mechanism of action is still not fully understood; the main mechanism hypothesized is COX inhibition (Hinz et al., 2008; Andersson et al., 2011). The method by which Paracetamol inhibits the COX-2 enzyme involves many interactions, including (H-bond, van der Waals, Hydrophobic, and pi-alkyl interactions), with the crucial amino acids in the binding pocket responsible for the COX-2 enzyme's activity Fig. 2

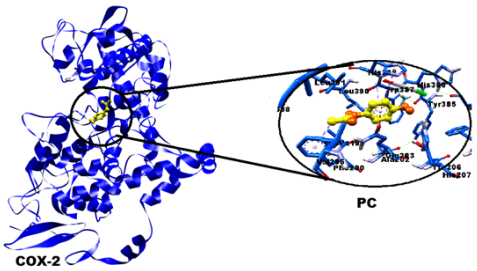

Fig. 2. Binding modes of Paracetamol compound inside the COX-2 receptor pocket.

Although paracetamol is safe for humans at a recommended dose (adults can take up to 4,000 mg per day in a single dose of 1,000 mg), high levels of paracetamol damage the liver and kidney, in addition to that, cause asthma and eczema in children under the age of one year (Daly et al., 2008; Behera et al., 2012). Paracetamol is used to treat severe pain, such as cancer discomfort and post-operative pain, in conjunction with opioid painkillers (Pasha, 2020) . Paracetamol has a very specific impact in the brain, blocking an enzyme that is involved in the transmission of pain. Although it provides systemic pain relief, it was known that its mode of action was different from that of other painkillers (Dixit and Patel 2014). It is on the WHO's List of Essential Medicines, which has the best and safest medicines a health care system (Pasha, 2020). Paracetamol is one of the most commonly used painkillers and medicines to fever reducers. Itis also used to ease mild to moderate pain from backaches, arthritis, headaches, toothaches, and pain after surgery (Iranifam et al., 2019; Moller et al, 2005). Also, it is an antipyretic used to treat viral and bacterial fevers. Although it relieves pain in different parts of the body in a manner that is distinct from that of other pain relievers, it was recognized that its mechanism of action was unique. It is considered one of the most efficient and risk-free treatments available, which is why the World Health Organization (WHO) included it on their list of essential medicines (Freo et al., 2021; Pasha, 2020; Ennis et al., 2016). Because of the widespread use of paracetamol and the adverse effects described earlier, the dosage of this medication must be accurately represented in pharmaceutical products like tablets. Determining the amount of active ingredient in a medicinal preparation is critical in the pharmaceutical industry to ensure a drug's quality, safety, and efficacy. If the amount of active ingredient does not match the amount listed on the label, the drug's efficacy or toxicity will be affected. Therefore, technique development continues in the hopes of achieving a quick, accurate, and effective alternate way. Numerous analytical methods for determining paracetamol in different pharmaceutical preparations have been reported, such as electrochemistry (Wang et al., 2018; Baccarin et al, 2017; Kachoosangi et al, 2008), Voltammetric (Bharathi et al., 2018; Kalambate et al, 2015; Yiğit et al., 2015), capillary electrophoresis (Zhao et al, 2006), HPLC (Chandra and Sharma, 2013; Abdelaleem et al., 2015; Topkafa et al., 2016; Merrikhi et al., 2016; Farid and Abdelaleem, 2016) and spectrophotometric (Dinç et al, 2002., Criado et al., 2000; Omar, 2014; Avasarala and Jayanthi, 2015; Ahmed et al., 2015; Desta and Amare, 2017; Saeed, 2017; Pasha, 2020).

The spectrophotometric detection of paracetamol is less selective and less sensitive than other methods, and several of the analytes involved are chronically hazardous and require particular experimental conditions. Therefore, this work aims to establish a validated method that is easy, time-saving, and cost-effective for quantitatively assessing paracetamol in its pharmaceutical tablet formulations using UV-Vis spectrophotometric analysis

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2. Materials and Methods

2.1 Experimental

All chemicals used are of analytical grade. Ethanol and Distilled water were used for preparing solutions. Paracetamol (PC) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). A commercial pharmaceutical preparation of Paracetamol in the form of tablets (500 mg) was purchased from a local pharmacy store. The brand information is shown in Table 1.

Code	Name	Dosage (mg)	Manufacturer	Exp. Date
A	Paracetamol actavisl (A)	500	ACTAVIS-England	2024
B	Ranadol Advice (B)	500	FUGEN-Ireland	2023
C	Paracetamol tablet PL HOLDER(C)	500	PL HOLDER-India	2026

2.2 Instrumentation

Utilizing a UV-1500 Spekol with 1 cm quartz cell, the spectrum was measured, and the validation characteristics were determined.

2.3 Standard preparation

Stock standard solution (100 µg mL^{-1}) of PC was prepared by dissolving 5 mg drug in 25 ml ethanol and was shaken well. Then, 25 ml of distilled water was added to adjust the volume to 50 ml. From that, 5 ml was taken, and the volume was adjusted up to 50 ml with diluents.

2.4 Test preparation

Ten tablets of each product were individually weighed and crushed into a powdery consistency. The powder corresponding to 10 mg of PC was weighed and dissolved in 50 mL of ethanol and sonicated for 10 minutes to increase the solubility. The residue was filtered through Whatman filter paper no. 41, transferred to a 100 ml standard flask and diluted to the mark with distilled water in order to generate a 100 µg mL-1. After filtering the sample, approximately 25 ml of the solution above was transferred to a 50 ml volumetric flask, and the volume was brought up to the mark with water.

From that, 10 µg mL-1 was prepared and introduced to the UV for the analysis

3.0 Results and Discussion

3.1 Selection of suitable solvent and wavelength

Solubility is one of the most essential physicochemical qualities of a medication. The solvent system should produce a homogenous solution where the investigated solute is entirely dissolved (Jouyban and Fakhree, 2012). Our first tests included various combinations of diluents: water with ethanol, methanol, and NaOH. The best results were achieved using a diluent composed of ethanol and water at a ratio of (50:50, v/v). In the range of 200-400 nm, the wavelength of the standard solution and solvent (ethanol: water) as the reference solution was scanned using a UV-VIS spectrophotometer shown in Fig. 3. 243nm was determined to be the wavelength corresponding to maximum absorption (λmax).

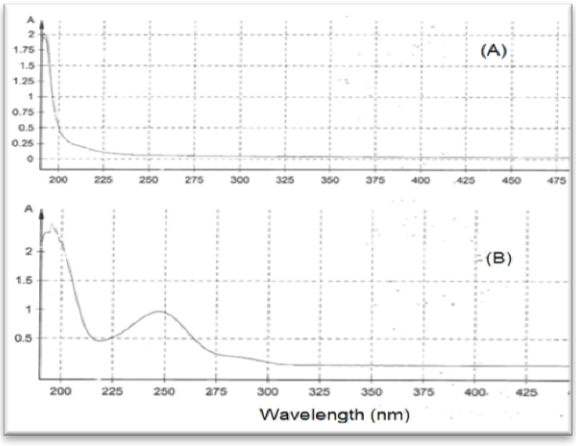

Fig. 3. UV-spectra of the (A) reference solution (B) standard solution

3.2 Validation of Analytical Method

3.2.1 Linearity, limit of detection (LOD) and limit of quantification (LOQ)

In quantitative analysis, linearity is a crucial requirement that constitutes a measure of accuracy over the range of the approach (Encarnação et al., 2020). Constructing a calibration curve using a total of eight standard solutions made it possible to assess the linearity of the suggested method. The linearity of the calibration plots was studied over a concentration range of 2.0–16.0 µg mL-1. The calibration curve was established by plotting the absorption (y) versus PC concentration (The calibration curve obtained was linear over the studied concentration range. The data analysis revealed a regression equation y=0.0754x+0.0216 with a correlation coefficient of 0.9997, indicating good linearity due to its high value Fig. 4.

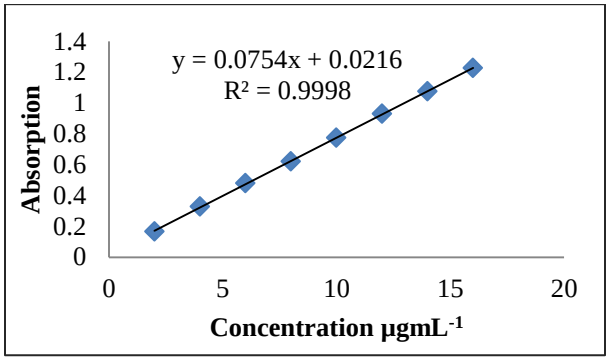

Fig. 4. Calibration curve of Paracetamol

The minimum amount of the analyte detected (LOD) is calculated using the equation "LOD=" 3.3S/K, where s is the standard deviation of the intercept and k is the slope of the calibration curve. Using this equation, the limit of detection was determined to be 0.5 µgml-1. The lowest analyte concentration detected with acceptable precision and accuracy is known as the limit of quantification (LOQ). It is derived using the equation" LOQ=" 10S/K Using this equation, the limit of detection was determined to be 1.5 µg mL-1.

3.2.3 Precision

The term "precision" refers to the degree to which the values being measured and those referenced agree with one another. Because precision is typically reliant on the concentration of the analyte, it is necessary to determine while working within the range of concentrations that are of interest. Repeatability (within-day precision) and intermediate precision (between-day precision) were measured for three consecutive days to evaluate precision (Magnusson, 2014). Three standard solution preparations were used to evaluate Intra-day precision at three concentration levels (2, 6 and 10 µg mL-1) on the same day (n = 9). Every concentration was measured thrice, while the inter-day precision was assessed over three consecutive days (n = 27). Table 2 shows that the calculated coefficient of variation for repeatability, intra-day, and inter-day results was within the permissible limit range (RSD% has not exceeded 2%) (Desta and Amare., 2017; Avasarala and Jayanthi, 2015). The results demonstrate the good repeatability of the method. Satisfactorily low coefficients of variation suggested a high precision level; therefore, the method developed could be used to determine PC in pharmaceutical formulations

Table 2. Shows the PC standard solution's intra-day and inter-day precision (%RSD).

PC concentration (µg mL^{-1})	RSD (%)		
Intra-dayprecision (n=9)	**0hr**	**1hr**	**2hr**
2	1.84	1.57	1.28
6	0.45	0.66	0.73
10	0.12	0.29	0.35
Inter-dayprecision (n=27)	**1st day**	**2nd day**	**3rd day**
2	1.8	0.46	0.78
6	0.22	0.56	0.85
10	1.76	1.93	1.54

3.2.4 Recovery

The accuracy of an analytical method can be defined as the degree to which the result of the value found is proximate to the reference value (FDA, 2001). The accuracy of the proposed method was determined by conducting recovery experiments at three different concentrations (2, 6 and 10 µg mL-1). Recoveries were performed by adding known amounts of PC to the pretreated formulations. Each concentration level was prepared in triplicates and introduced to the UV/Visible thrice. The results obtained are summarized in Table 3. The outcome demonstrated that the approach was accurate as the best recoveries (103-109%) of the spiked drug were obtained at each additional concentration; this falls within the recommended recovery percentage range of 80 to 110%, indicating this method's potential for determining the PC in tablet dosage formulations.

Table 3. Recovery of the proposed method.

Sample (µg mL^{-1})	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery ± RSD(%)*
6	2	8.06	103±1.62
6	6	12.44	107±1.63
6	10	16.9	109±1.28

3.2.5 Analysis of pharmaceutical formulation

In order to determine PC in three commercially available formulations, the validated method was successfully used. The results are based on the average of three determined values obtained are shown in Table 4. According to what can be seen in this table, no significant differences were identified between the values of paracetamol on the label (data supplied by the manufacturer) and the values that were found (data obtained by the proposed method) in the B and C samples. Based on the USP pharmacopoeia, the paracetamol concentration must be between 90% and 110% of the labelled amount (USP, 2018). As a result, the two brands studied B and C, complied with USP pharmacopeial requirements, while brand A was higher than the standard, which is hazardous to the community

Table 4. Assay results of PC in different pharmaceutical formulation.

Brand	Amount of drug labelled (mg)	Amount of drug estimated (mg)	% Labelled claim±RSD
paracetamol actavisl (A)	500	625	124.6±0.413
RANADOL ADVICE (B)	500	514	102.7±0.083
paracetamol tablet PL HOLDER (C)	500	503	100.5±0.448

4. Conclusion

The conclusion is that the current analytical procedure was verified in accordance with the ICH standard and has satisfied particular acceptance requirements. The proposed method was found to be linear, accurate, precise, and sensitive. The suggested method is suitable for the routine analysis of paracetamol in pharmaceutical dosage formulations, as shown by the good recoveries and low coefficient of variation. Based on the findings of this research, brands B and C of paracetamol were compliant with the USP criteria, while brand A exceeded the criterion.

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