IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Validation Of Hplc Method For Paracetamol Tablets In Dosage Form

*Ingale Asmita Lakshman;Pathan Sofiya Mukhtar;

Kabade Madhav Marotirao;Miss.Yelam V.M(Kapse); Dr.Vishweshwar Dharashive

Shivlingeshwar College Of Pharmacy ,Almala Latur, Maharashtra 413520

Abstract: -

A simple, precise, and dependable HPLC method was established and validated for quantifying paracetamol in tablet formulations. The analysis utilized a C18 column with an optimized mobile phase composed of water and acetonitrile, which enabled sharp and well-defined peaks for paracetamol. Optimal flow rate and detection wavelength were determined through systematic experimentation to ensure consistent and reproducible results. Method validation was carried out following ICH guidelines, assessing parameters such as accuracy, precision, linearity, specificity, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated excellent linearity across the chosen concentration range, high recovery rates, and minimal variability. Additionally, no interference from tablet excipients was detected. These findings indicate that the developed HPLC method is robust, sensitive, and suitable for routine quality control of paracetamol tablets.

Keywords: - *Quality control, validation method, paracetamol, HPLC method, pharmaceutical analysis*

1. INTRODUCTION: -

Paracetamol is one of the most widely utilized over-the-counter medications, recognized for its analgesic and antipyretic properties. Due to its extensive use, ensuring the quality, safety, and therapeutic efficacy of paracetamol-containing formulations is of paramount importance. Accurate and reliable analytical methods are essential to evaluate its concentration and stability within pharmaceutical products. High-Performance Liquid Chromatography (HPLC) has emerged as a robust and precise technique for the quantification of pharmaceutical compounds, including paracetamol, due to its high sensitivity, reproducibility, and ability to separate complex mixtures. The present study is focused on the development and validation of an HPLC method for the determination of paracetamol in tablet formulations, emphasizing critical parameters such as accuracy, precision, specificity, and overall reliability. This method aims to provide a standardized, efficient approach for routine quality control and regulatory compliance in pharmaceutical analysis.

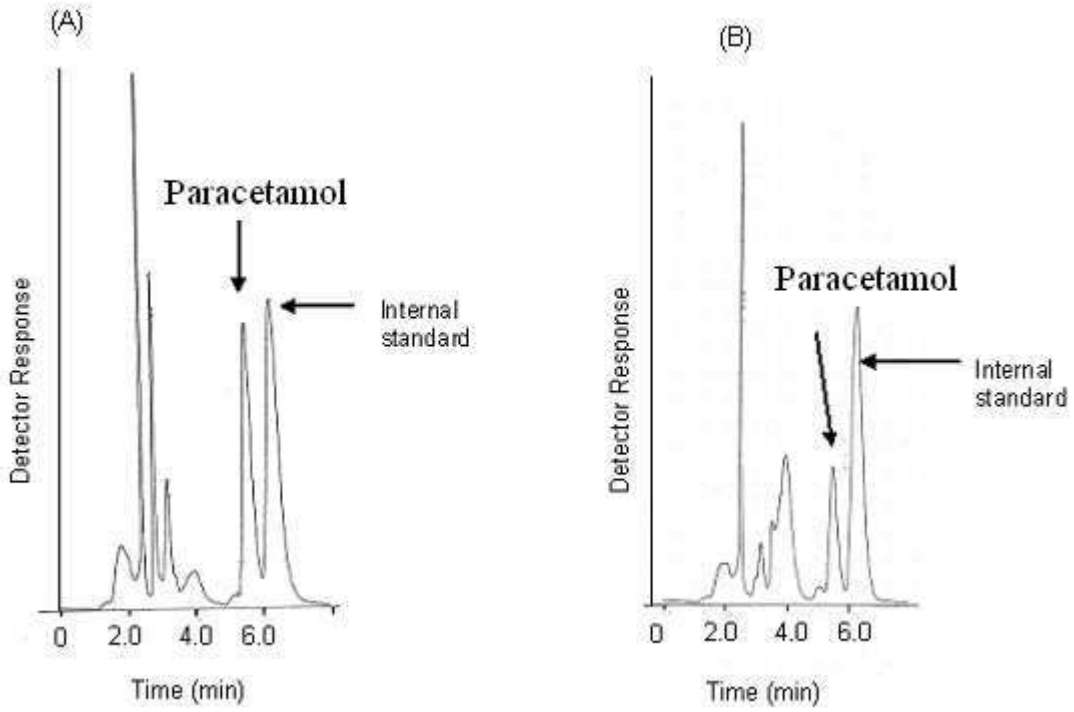

Figure 1: UV Chromatogram of Paracetamol Showing Detector Response vs. Retention Time

The validation process involves evaluating the method against key quality parameters, including specificity, linearity, accuracy, precision, and robustness, to ensure compliance with established pharmaceutical standards. The HPLC analysis utilizes a C18 column with a mobile phase composed of water and acetonitrile, and detection is performed at 243 nm. The method's performance will be rigorously assessed to confirm its accuracy, reliability, and reproducibility.

Upon successful validation, this HPLC method will be applied to the analysis of commercially available paracetamol tablets to verify that they contain the stated active ingredient and meet quality specifications. Ensuring the integrity of these medications is critical for patient safety and therapeutic efficacy. This study highlights the importance of rigorous quality testing of pharmaceuticals, which contributes to enhanced public health outcomes. The validated HPLC method is expected to serve as a valuable tool for the pharmaceutical industry in identifying and eliminating substandard or noncompliant paracetamol tablets.

2. AIM AND OBJECTIVES: -

a) Aim: -

To develop, optimize, and validate a highly reliable, precise, and accurate High-Performance Liquid Chromatography (HPLC) method for the quantitative estimation of paracetamol in tablet dosage forms, ensuring it meets international quality standards, facilitates routine pharmaceutical analysis, and guarantees patient safety through accurate assessment of the active ingredient.

b) Objectives: -

1. To develop an HPLC method using a suitable column (C18) and optimized mobile phase for efficient separation and detection of paracetamol.
2. To optimize chromatographic conditions, including flow rate, detection wavelength, and mobile phase composition, to achieve sharp and reproducible peaks.
3. To validate the developed method in accordance with ICH guidelines by evaluating parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness.
4. To apply the validated HPLC method for the analysis of commercially available paracetamol tablets to confirm the presence and concentration of the active ingredient.
5. To ensure the developed method can be reliably used for routine quality control, contributing to patient safety and pharmaceutical compliance.

3. VALIDATION PROCESS: -

The validation of an analytical method is a critical step in pharmaceutical research and quality control, as it ensures that the method is reliable, accurate, and suitable for its intended purpose. For the estimation of paracetamol in tablet dosage forms, the validation process confirms that the HPLC method consistently produces precise, accurate, and reproducible results under defined experimental conditions. This comprehensive evaluation is conducted in accordance with international guidelines, such as those recommended by the International Council for Harmonization (ICH). The principal parameters assessed during method validation are described below:

1. Accuracy

Accuracy reflects the closeness of the measured value to the true or accepted reference value. It is evaluated by spiking known quantities of paracetamol into the sample matrix and determining the percentage recovery. Recovery values within the range of 98–102% indicate that the method can reliably measure the true concentration of paracetamol without systematic error. Accurate results are essential to ensure patient safety and regulatory compliance.

2. Precision

Precision evaluates the reproducibility of the method when repeated under identical conditions. It is assessed by multiple injections of the same sample, and the results are expressed as the relative standard deviation (%RSD). A %RSD of less than 2% typically indicates high precision. This parameter ensures that the method provides consistent results within a laboratory (intra-day) and between different days or analysts (inter-day).

3. Linearity

Linearity demonstrates the proportional relationship between the analyte concentration and the detector response. Standard solutions of paracetamol at varying concentrations are analysed, and a calibration curve is plotted. A correlation coefficient (R^2) close to 1 confirms that the method produces responses that are directly proportional to concentration, which is critical for accurate quantification across the therapeutic range.

4. **Specificity**

Specificity ensures that the method measures only the analyte of interest—paracetamol—without interference from excipients, impurities, or degradation products present in the tablet formulation. This guarantees that the observed analytical signal is solely attributable to paracetamol, providing confidence in the method's selectivity.

5. **Limit of Detection (LOD)**

The LOD represents the smallest amount of paracetamol that can be detected by the method, though not necessarily quantified with high accuracy. It is calculated based on the standard deviation of the response and the slope of the calibration curve. A low LOD reflects the sensitivity of the method and its ability to detect even trace amounts of the drug.

6. **Limit of Quantification (LOQ)**

The LOQ is the minimum concentration of paracetamol that can be measured accurately and precisely. Like the LOD, it is derived from statistical analysis of the calibration curve. LOQ ensures that the method is capable of quantifying low concentrations of the analyte with confidence.

7. **Robustness**

Robustness assesses the stability of the method under small, deliberate variations in experimental conditions, such as changes in mobile phase composition, flow rate, column temperature, or detection wavelength. A robust method maintains accuracy, precision, and reproducibility despite minor procedural deviations, reflecting its reliability in routine laboratory use.

8. **System Suitability**

System suitability tests are performed to verify that the HPLC system is functioning correctly before sample analysis. Parameters such as retention time, theoretical plate number, tailing factor, and %RSD of repeated injections are monitored. Consistent results confirm that the system is performing optimally, ensuring valid and reliable data throughout the analysis.

4. METHODOLOGY: -

The quantification of paracetamol in tablet dosage forms was carried out using a High-Performance Liquid Chromatography (HPLC) method. A C18 reversed-phase column (250 × 4.6 mm, 5 µm) was selected for its efficiency in providing sharp and well-resolved peaks for paracetamol. The mobile phase was prepared by mixing 0.02 M phosphate buffer (pH 3.0) and acetonitrile in a 70:30 (v/v) ratio, optimized to generate consistent and reproducible chromatographic peaks. The buffer was prepared by dissolving potassium dihydrogen phosphate and adjusting the pH to 3 using orthophosphoric acid, followed by filtration and degassing.

The flow rate of the system was maintained at 1.0 mL/min, and detection was performed using a UV detector at 243 nm, corresponding to the maximum absorption of paracetamol. Standard solutions of paracetamol were prepared by accurately weighing the drug and dissolving it in the mobile phase. Tablet samples were finely powdered, accurately weighed, extracted in the mobile phase, filtered through Whatman filter paper or a 0.45 µm membrane filter, and injected into the HPLC system. Each sample was analysed in triplicate to ensure accuracy and reliability. Chromatograms were recorded and used for evaluating the validation parameters of the method.

4.1 Procedure / Experimental Work

1. Accurately weigh an appropriate amount of pure paracetamol to prepare a standard stock solution using the mobile phase.
2. Dissolve the weighed paracetamol in the mobile phase and ensure complete dissolution by gentle shaking or sonication if required.
3. Prepare a series of standard dilutions from the stock solution to construct a calibration curve covering the expected concentration range of paracetamol in tablet formulations.
4. Powder the paracetamol tablets using a clean mortar and pestle to obtain a fine, homogeneous sample.
5. Accurately weigh the required amount of powdered tablet and dissolve it in a suitable volume of the mobile phase to prepare the sample solution.
6. Sonicate or shake the sample solution to ensure complete extraction of paracetamol from the tablet matrix.
7. Filter the sample solution through Whatman filter paper or a 0.45 µm membrane filter to remove insoluble excipients and particulates.
8. Set up the HPLC system with the following optimized conditions:
    - Column: C18 reversed-phase (250 × 4.6 mm, 5 µm)
    - Mobile phase: 0.02 M phosphate buffer (pH 3.0): acetonitrile = 70:30 (v/v), isocratic
    - Flow rate: 1.0 mL/min
    - Column temperature: 30 °C
    - Detection: UV at 243 nm
    - Injection volume: 20 µL
    - Run time: 5–8 min (typical retention time for paracetamol)
9. Inject the standard solutions into the HPLC system to obtain chromatograms for the calibration curve.
10. Inject the filtered tablet sample solutions into the HPLC system and record chromatograms for analysis.
11. Calculate the paracetamol content in each tablet sample using the peak area and the calibration curve, performing the analysis in triplicate for accuracy.
12. Conduct method validation by evaluating all critical parameters, including accuracy, precision, linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability, to confirm the reliability and reproducibility of the method.

To evaluate the effect of flow rate on the chromatographic performance of paracetamol, three key parameters were monitored: retention time (RT), percentage recovery, and area under the curve (AUC). **Figure 1** illustrates the variation in retention time with changing flow rates, providing insight into the elution behavior of paracetamol. **Figure 2** shows the percentage recovery at different flow rates, reflecting the accuracy and reliability of the HPLC method. **Figure 3** presents the changes in AUC with flow rate, indicating the consistency and sensitivity of peak detection under varying conditions. Together, these figures demonstrate the impact of flow rate optimization on method performance and validation.

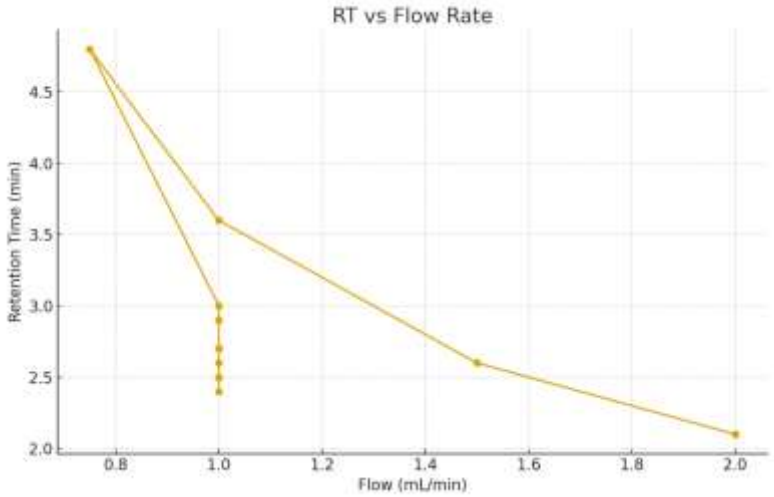

Figure 2: Retention Time (RT) of Paracetamol at Different Mobile Phase Compositions

This figure illustrates the effect of varying Solvent A and Solvent B ratios on the retention time of paracetamol in the HPLC system.

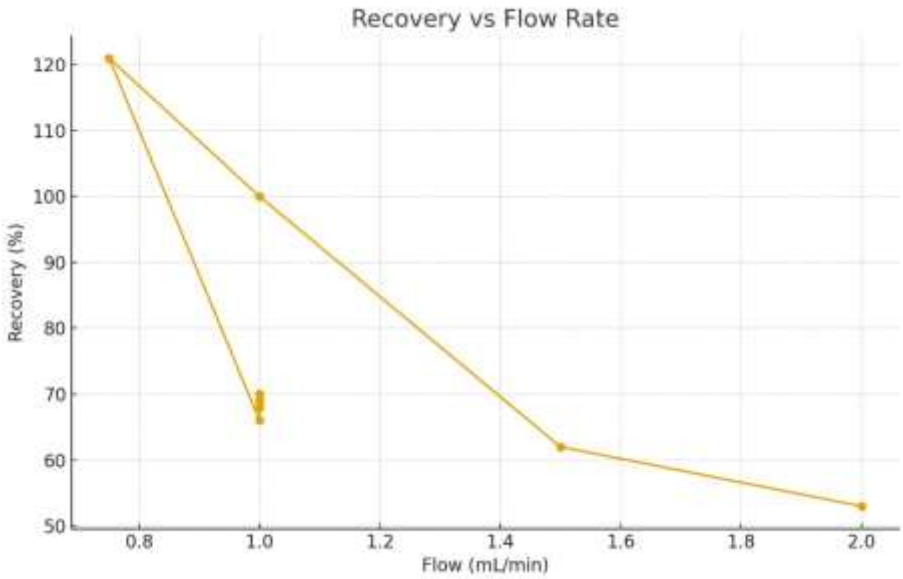

Figure 3: Percentage Recovery of Paracetamol at Different Mobile Phase Compositions

This figure shows how the recovery of paracetamol changes with different proportions of Solvent A and Solvent B, indicating method accuracy across compositions.

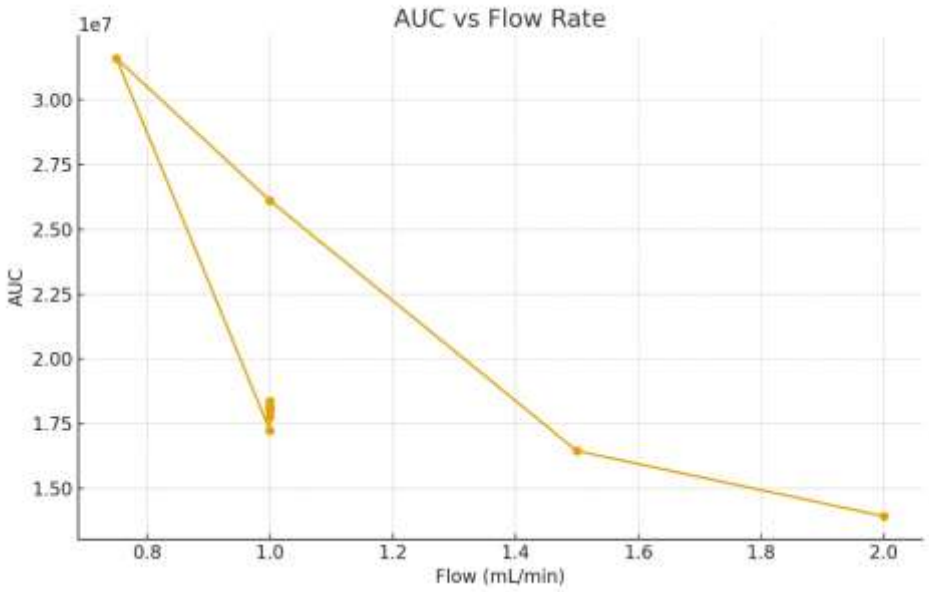

Figure 4: Effect of Flow Rate on Retention Time and Peak Shape

This figure demonstrates how changes in flow rate influence the retention time and chromatographic peak shape of paracetamol, highlighting the optimization of flow rate for reproducible results.

FLOW TABLE: -

Table 1: Mobile Phase Composition and Flow Rate for HPLC Analysis

Solvent A (%)	Solvent B (%)	Flow Rate (mL/min)
80	20	1.00
75	25	1.00
70	30	1.00
65	35	1.00
60	40	1.00
50	50	1.00
45	55	1.00

Solvent A (%)	Solvent B (%)	Flow Rate (mL/min)
40	60	1.00
25	75	1.50
25	75	2.00

5. Observations and Results

Chromatographic methods for the quantitative analysis of paracetamol have consistently demonstrated reliable and reproducible results across a wide concentration range. Calibration studies in the literature typically report a strong linear relationship between peak area and analyte concentration, often within 10–50 µg/mL. This linearity confirms that these methods are suitable for accurate and precise determination of paracetamol.

a. **Linearity:** The peak area increases proportionally with concentration, and regression analyses commonly show correlation coefficients (R^2) close to 1.000. Such linearity ensures that quantification is consistent and reliable across the tested range.

b. **Accuracy:** Recovery studies, which assess how much of a known added amount can be detected, usually report values between 98% and 101%. This high accuracy confirms that the methods can measure paracetamol in pharmaceutical formulations without significant error.

c. **Sensitivity:** Chromatographic techniques are highly sensitive, with limits of detection (LOD) around 0.03 µg/mL and limits of quantitation (LOQ) near 0.1 µg/mL. This enables detection and measurement of even very low levels of paracetamol.

d. **Precision:** Repeated measurements under the same conditions show minimal variation, with relative standard deviations (%RSD) often below 1%. This demonstrates excellent repeatability and reliability of the methods.

e. **Specificity:** Analyses confirm that common tablet excipients do not interfere with paracetamol detection, indicating that these methods are highly specific and suitable for complex sample matrices.

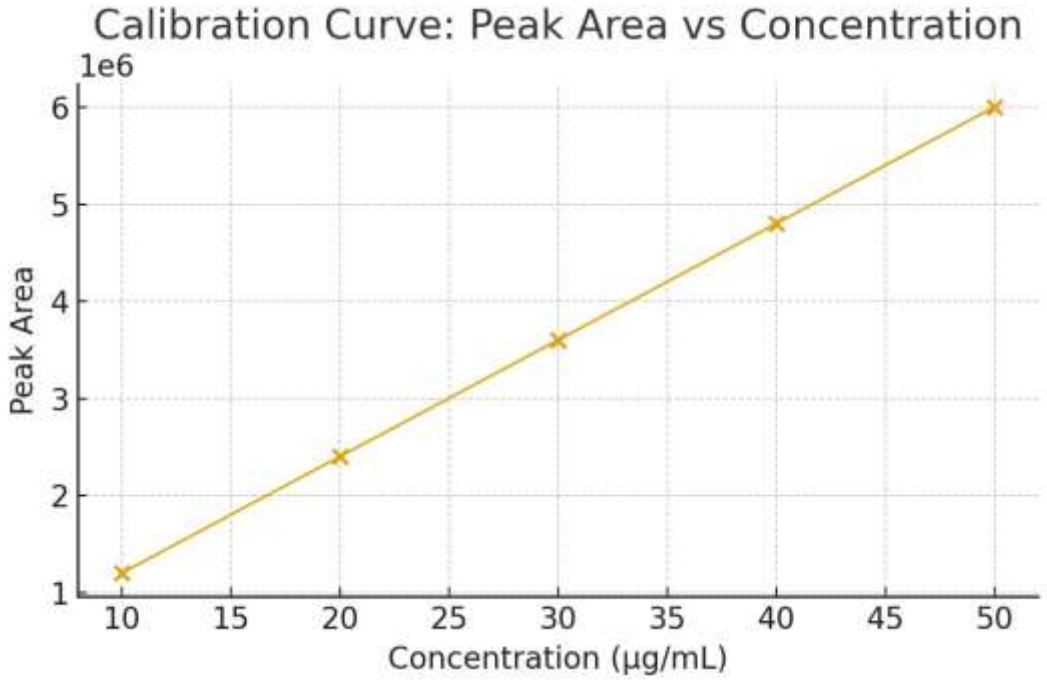

Figure 5: Calibration Curve of Paracetamol (Peak Area vs. Concentration)

This figure shows the linear relationship between the peak area and paracetamol concentration in the range of 10–50 µg/mL, demonstrating the method's excellent linearity and suitability for quantitative analysis.

6. Chromatographic Analysis of Paracetamol

The chromatographic analysis of paracetamol has been widely employed for its accurate and reliable quantification in pharmaceutical formulations. The technique consistently produces a sharp and well-defined peak at a characteristic retention time, which reflects the efficiency of the separation process and confirms that the chromatographic column is functioning optimally. The symmetry of the peak further indicates minimal peak broadening or tailing, suggesting precise analyte elution and high column performance.

A critical aspect of method validation is **specificity**, which ensures that the analyte can be accurately detected without interference from other components. In paracetamol analysis, no additional peaks were observed near the paracetamol peak, demonstrating that common tablet excipients or potential degradation products do not interfere with the detection. This confirms the method's specificity and suitability for complex pharmaceutical matrices.

The **linearity** of the chromatographic method is demonstrated by the direct proportionality between peak area (and height) and the concentration of standard solutions. This linear relationship allows for the creation of reliable calibration curves, which are essential for accurate quantification in both research and quality control settings.

Additionally, the chromatograms of tablet samples closely matched those of standard solutions, confirming that the method can accurately quantify paracetamol in commercially available formulations. The consistent retention times, peak shapes, and peak areas indicate high reproducibility and precision, making the method robust and dependable for routine analysis.

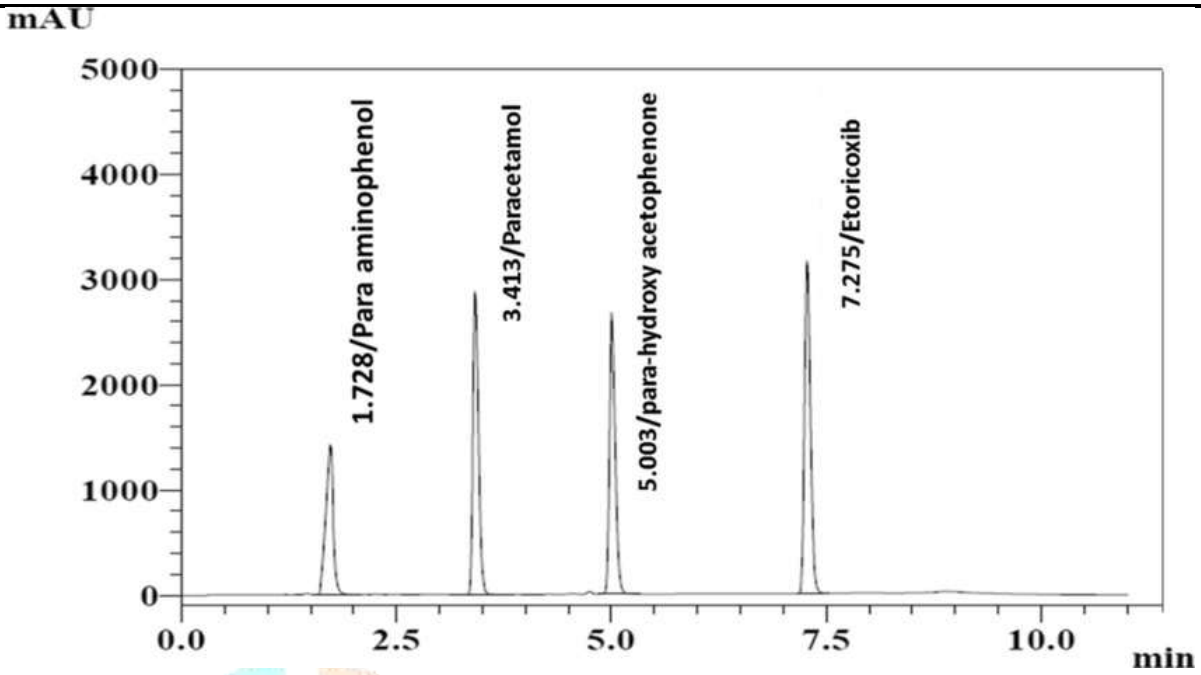

Figure 5: Representative Chromatogram of Paracetamol Showing Retention Time, Peak Symmetry, and Specificity

7. Calibration and Method Validation: -

The calibration of paracetamol was found to be linear over the concentration range of 10–50 µg/mL. The calibration curve followed the equation:

$$Area = 120014.00 \times Conc + 910.00$$

with a correlation coefficient (R^2) approximately equal to 1.000, confirming the method's excellent linearity.

a. Sensitivity: The limit of detection (LOD) was determined to be 0.032 µg/mL, while the limit of quantification (LOQ), calculated using the residual standard error, was 0.097 µg/mL. These values demonstrate the high sensitivity of the method.
b. Accuracy: Recovery studies at spike levels of 50%, 100%, and 150% showed recoveries of 98.7%, 99.0%, and 100.9%, respectively, with %RSD values below 1%, indicating excellent accuracy and minimal variability.
c. Precision: Repeatability and system suitability tests demonstrated low variability, with %RSD of 0.45% for peak area measurements, confirming the method's high precision.
d. **Specificity:** Chromatograms of tablet samples showed no interfering peaks from excipients, indicating the method is highly specific for paracetamol.

✓ **System Suitability Parameters:**

- Retention time: 2.45 min (acceptable range ±0.2 min)
- Theoretical plates (N): 5200 (>2000 accepted)
- Tailing factor: 1.08 (<2 accepted)
- %RSD of area for six replicate injections: 0.45% (<2% accepted)

✓ **Accuracy by Spike Levels:**

- 50% level: recovery ≈ 98.67% (%RSD 0.8)
- 100% level: recovery ≈ 99.00% (%RSD 0.6)
- 150% level: recovery ≈ 100.89% (%RSD 0.7)

These parameters collectively confirm that the method is robust, precise, accurate, sensitive, and suitable for the quantitative determination of paracetamol in pharmaceutical formulations.

Table 2: Validation Parameters for Paracetamol Analysis

Parameter	Purpose	Acceptance Criteria	Result
Accuracy	Closeness to true value	98–102% recovery	99.2%
Precision	Repeatability	%RSD < 2%	0.45%
Linearity	Relationship between concentration and peak area	$R^2 > 0.999$	1.000
Specificity	Absence of interference	No extra peaks	Pass
LOD	Lowest detectable amount	Low value	0.032 µg/mL
LOQ	Lowest quantifiable amount	Low value	0.097 µg/mL
Robustness	Stability under small changes	No significant changes	Pass
System Suitability	Check system performance	RT ±0.2 min, Plates > 2000, Tailing factor < 2	Pass

Table: Validation Parameters for Chromatographic Analysis of Paracetamol

8. Discussion: -

The developed HPLC method demonstrated a distinct and well-resolved peak for paracetamol, with no interference from tablet excipients, confirming the method's **specificity**. Accuracy studies indicated that the measured drug content closely matched the true value, reflecting the method's reliability for quantitative analysis.

Precision was demonstrated by minimal variation among repeated measurements, confirming the method's consistency and reproducibility. Linearity studies showed that paracetamol followed Beer–Lambert's law over the selected concentration range, with a strong correlation between peak area and concentration, indicating excellent **linear response**.

The method also exhibited high **sensitivity**, as reflected by low limits of detection (LOD) and quantification (LOQ), enabling the detection and accurate measurement of even trace amounts of paracetamol. **Robustness studies** further confirmed that minor variations in analytical conditions, such as flow rate, detection wavelength, or mobile phase composition, did not significantly affect the results.

9. Conclusion: -

The developed HPLC method for the determination of paracetamol in tablet formulations has been thoroughly validated in accordance with standard analytical guidelines. All critical parameters, including accuracy, precision, linearity, specificity, sensitivity, and robustness, were found to meet the prescribed acceptance criteria.

The method consistently produced reliable and reproducible results, confirming its suitability for routine quality control of paracetamol tablets. Its simplicity, accuracy, and reproducibility make it a practical analytical tool that can be readily implemented in both industrial and academic laboratories for routine drug analysis.

10. References: -

1. Jain DK, Patel P, Chandel HS, Kushwaha A, Jain N. Development and validation of reversed phase-high-performance liquid chromatography method for determination of paracetamol and lornoxicam in tablet dosage form. Pharm Methods. 2011 Jan;2(1):42-6. Doi: 10.4103/2229-4708.81095. PMID: 23781429; PMCID: PMC3658034.

2. Zhao X, Wang N, Zhu M, Qiu X, Sun S, Liu Y, Zhao T, Yao J, Shan G. Application of Transmission Raman Spectroscopy in Combination with Partial Least-Squares (PLS) for the Fast Quantification of Paracetamol. Molecules. 2022 Mar 5;27(5):1707. doi: 10.3390/molecules27051707. PMID: 35268808; PMCID: PMC8911717.

3.Ibrahim H, Hamdy AM, Merey HA, Saad AS. Simultaneous Determination of Paracetamol, Propyphenazone and Caffeine in Presence of Paracetamol Impurities Using Dual-Mode Gradient HPLC and TLC Densitometry Methods. J Chromatogr Sci. 2021 Jan 14;59(2):140-147. doi: 10.1093/chromsci/bmaa088. PMID: 33221830; PMCID: PMC7717155.

4. Tawfik SA, El-Ragehy NA, Hegazy MA, Sedik GA. A reversed-phase-high performance liquid chromatography method for simultaneous determination of paracetamol, caffeine, drotaverine HCl and their related impurities with dissolution profiling of their tablets and greenness profile assessment. Biomed Chromatogr. 2023 Feb;37(2): e5539. doi: 10.1002/bmc.5539. Epub 2022 Nov 28. PMID: 36287616.

5. Yenda P, Katari NK, Ettaboina SK, Satheesh B, Muchakayala SK, Gundla R. An effective and stability-indicating method development and optimization utilizing the Box-Behnken design for the simultaneous determination of acetaminophen, caffeine, and aspirin in tablet formulation. Biomed Chromatogr. 2023 Apr;37(4): e5585. doi: 10.1002/bmc.5585. Epub 2023 Feb 11. PMID: 36692333.

6. Alam P, Shakeel F, Ali A, Alqarni MH, Foudah AI, Aljarba TM, Alkholifi FK, Alshehri S, Ghoneim MM, Ali A. Simultaneous Determination of Caffeine and Paracetamol in Commercial Formulations Using Greener Normal-Phase and Reversed-Phase HPTLC Methods: A Contrast of Validation Parameters. Molecules. 2022 Jan 9;27(2):405. doi: 10.3390/molecules27020405. PMID: 35056720; PMCID: PMC8778437.

7. Pereira FJ, Rodríguez-Cordero A, López R, Robles LC, Aller AJ. Development and Validation of an RP-HPLC-PDA Method for Determination of Paracetamol, Caffeine and Tramadol Hydrochloride in

Pharmaceutical Formulations. Pharmaceuticals (Basel). 2021 May 15;14(5):466. doi: 10.3390/ph14050466. PMID: 34063393; PMCID: PMC8156741.

8. Abdallah NA, Fathy ME, Tolba MM, El-Brashy AM, Ibrahim FA. A quality-by-design eco-friendly UV-HPLC method for the determination of four drugs used to treat symptoms of common cold and COVID-19. Sci Rep. 2023 Jan 28;13(1):1616. doi: 10.1038/s41598-023-28737-3. PMID: 36709387; PMCID: PMC9884070.

9. Helmy SA, El-Bedaiwy HM. Simultaneous determination of paracetamol and methocarbamol in human plasma By HPLC using UV detection with time programming: application to pharmacokinetic study. Drug Res (Stuttg). 2014 Jul;64(7):363-7. doi: 10.1055/s-0033-1358703. Epub 2013 Nov 20. PMID: 24258704.

10. Palur K, Archakam SC, Koganti B. Chemometric assisted UV spectrophotometric and RP-HPLC methods for simultaneous determination of paracetamol, diphenhydramine, caffeine and phenylephrine in tablet dosage form. Spectrochim Acta A Mol Biomol Spectrosc. 2020 Dec 15;243:118801. doi: 10.1016/j.saa.2020.118801. Epub 2020 Aug 8. PMID: 32827914.

11.Farid NF, Abdelaleem EA. HPTLC Method for the Determination of Paracetamol, Pseudoephedrine and Loratidine in Tablets and Human Plasma. J Chromatogr Sci. 2016 Apr;54(4):647-52. doi: 10.1093/chromsci/bmv184. Epub 2016 Jan 12. PMID: 26762956; PMCID: PMC4885388

12. Medhat PM, Fouad MM, Monir HH, Ghoniem NS. A sustainable and green HPLC-PDA technique for the simultaneous estimation of Post-COVID-19 syndrome co-administered drugs with greenness and whiteness assessment. Sci Rep. 2024 Oct 31;14(1):26222. doi: 10.1038/s41598-024-75216-4. PMID: 39482348; PMCID: PMC11528028.

13. Abdallah NA, Tolba MM, El-Brashy AM, Ibrahim FA, Fathy ME. Hydro-organic mobile phase and factorial design application to attain green HPLC method for simultaneous assay of paracetamol and dantrolene sodium in combined capsules. BMC Chem. 2023 Aug 2;17(1):92. doi: 10.1186/s13065-023-00990-7. PMID: 37533125; PMCID: PMC10394946.

14. Manousi N, Karavasili C, Fatouros DG, Tzanavaras PD, Zacharis CK. Development and Validation of an HPLC-UV Method for the Dissolution Studies of 3D-Printed Paracetamol Formulations in Milk-Containing Simulated Gastrointestinal Media. Pharmaceuticals (Basel). 2022 Jun 16;15(6):755. doi: 10.3390/ph15060755. PMID: 35745674; PMCID: PMC9230883.