IJPSR (2026), Volume 17, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 October 2025; received in revised form, 28 October 2025; accepted, 02 November 2025; published 01 March 2026

COMPARATIVE PERFORMANCE OF LIQUID CHROMATOGRAPHY AND SPECTROPHOTOMETRY FOR QUANTITATIVE ANALYSIS OF PARACETAMOL IN TABLETS FORMULATIONS

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Keywords:

Paracetamol, Liquid Chromatography, Spectrophotometry, Quantitative Analysis, Validation, % Assay, UHPLC

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ABSTRACT: Several techniques have been developed and validated for analyzing paracetamol in pharmaceutical formulations, including Ultra High-Performance Liquid Chromatography (UHPLC). This study employed a validated method for analyzing three commercially available paracetamol products. The chromatographic separation utilized a mobile phase consisting of a 70:30 v/v ratio of methanol to water. The separation process was carried out using a C18 Rapid Resolution column (4.6 x 100 mm, 3.5 µm, Agilent HPLC Column). The maximum wavelength (λmax) for paracetamol estimation was determined at 243 nm. The separation process was achieved using an injection volume of 20 µl and a run time of 3.0 minutes. The method exhibited linearity within the concentration range of 10-150 µg/ml for Paracetamol. The validation of the method followed the International Conference on Harmonization (ICH) guidelines, covering assessments of specificity, selectivity, linearity, accuracy, and precision, as well as the lower limit of quantification (LLOQ), and lower limit of detection (LLOD). The LLOQ and LLOD for paracetamol were established at 250 ng/ml and 100 ng/ml, respectively. The assay percentage for all pharmaceutical products containing paracetamol met the acceptable criteria range of 90-110%. These methodologies have proven effective for the accurate quantitative analysis of paracetamol in pharmaceutical formulations.

INTRODUCTION: Paracetamol, also known as acetaminophen or 4-acetamidophenol, is a widely used analgesic and antipyretic.

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DOI:
10.13040/IJPSR.0975-8232.17(3).1025-32

This article can be accessed online on
www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(3).1025-32

In 1878, an American chemist named Harmon Northrop Morse synthesized paracetamol by reducing p-nitrophenol with tin in glacial acetic acid.

However, the therapeutic potential of paracetamol wasn't recognized and widely used until much later in the 20th century. Unlike NSAIDs, paracetamol does not have significant anti-inflammatory properties. Paracetamol, available over-the-counter,

is the primary alternative for individuals with sensitivity to aspirin (acetylsalicylic acid) [1]. It is often combined with decongestants, antihistamines, cough suppressants, or expectorants for treating cold or flu [2].

The literature describes several methods and techniques for determining paracetamol in various pharmaceutical preparations. These methods include titrimetric [3, 4], high-performance liquid chromatography (HPLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) [5, 6, 7], high-performance thin layer chromatography (HPTLC) [8, 9], voltammetry [10, 11, 12, 13], electrochemical analysis [14], and spectrophotometry [15, 16], all of which have been reported and validated. Nonetheless, the spectrophotometric techniques employed in determining paracetamol can often lack sensitivity, and selectivity, and may pose toxicity risks [17, 18, 19, 20].

Reliable, robust, and safe analytical techniques are pivotal for the analysis and detection of paracetamol, both for quality control in pharmaceutical formulations and subsequently for medical monitoring in biological fluids. Therefore, the ability to accurately quantify paracetamol in different settings is essential. The present research aims to evaluate the sensitivity and specificity of the Ultra High Performance Liquid Chromatography (UHPLC) technique for the quantitation of paracetamol in both pure and pharmaceutical dosage forms to establish the most sensitive methods.

MATERIALSAND METHODS:

Chemicals: Paracetamol standard powder (Paracetamol, Molekula®, UK) was used to prepare the standard stock solution and serial dilutions. Methanol CHROMASOLV™, for HPLC, with a purity of ≥ 99.9%, was obtained from Honeywell (USA).Ultra-pure water system (Milli-Q® Integral 5 (ZRXQ005T0) Millipore®) (USA), was used as HPLC grade water.

UHPLC Analysis: An Agilent 1290 Infinity model UHPLC equipped with a Variable Wavelength Detector (VWD) was used. Data acquisition, recording, and chromatographic integration were performed using the Open labEZ Chromsoftware (version A.01.05), for method development and validation of paracetamol. Isocratic elution was performed using an aqueous mobile phase consisting of a mixture of methanol and water (70:30, v/v). The mobile phase was filtered through a 0.45 µm pore size filter, followed by sonication and degassing in an ultrasonic bath (CPX5800H-E, Bransonic®). As Aljeboree *et al.*[21] outlined, a standard paracetamol solution was prepared by dissolving 1000 mg of the powder in Milli-Q water and gently shaken to ensure complete dissolution, yielding stock solution of 1.00 mg/ml. The stock standard solution of paracetamol (1.00 mg/ml) was diluted with water to prepare calibration standard solutions ranging from 10 to 150 µg/ml, up to a total volume of 100 ml. The standard solution was then transferred into a 2 ml glass vial (Thermo Fisher Scientific Inc.) and injected into the UHPLC system [21].

According to the method described by Saeed *et al.* [20] for the preparation of pharmaceutical samples for assay tests of tablet formulations, the average weight of ten tablets from each of the following products was determined: Panadol®, Panadol Cold & Flu®, and Panadol Extra. Each set of tablets was then finely crushed into a powder, and an amount equivalent to the average weight of the tablet was transferred to a 500 ml amber volumetric flask, diluted with water, and gently shaken to ensure the homogeneity of the solution. To standardize the paracetamol concentration across all samples, the assay test solutions were prepared to a concentration of 1.00 mg/ml.

These solutions were filtered immediately using a syringe (BD 10 ml, Luer-Lok™ Tip) fitted with a filter (Nalgene™ syringe filter 25 mm (0.45 µm) PES, Thermo Fisher) into a 10 ml glass tube. Next, 10 ml of the sample was pipetted using the (Micropipette 1000 µl, Eppendorf Research® plus) into a 100 ml amber volumetric flask, diluted to the mark with water, and gently shaken resulting in a final concentration of 100.0 µg/ml. Three replicate solutions were prepared for analysis, labeled and 20 µl of each sample was injected into the UHPLC system [20].

Reverse-Phase Ultra-High-Performance Liquid Chromatography (RP-UHPLC) Method Development, Optimization, and Validation: The RP-HPLC was carried out using ZORBAX Eclipse

Plus C18 Rapid Resolution Agilent HPLC Column (4.6 x 100 mm, 3.5 µm) at room temperature. The detection wavelength was set at λmax of 243 nm using anisocratic mobile phase consisting of a mixture of methanol and water in a 70:30 ratio(v/v) delivered at a flow rate of 1.00 ml/min, following the method described by Youssef *et al* [22].

The chromatographic separation was performed with an injection volume of 20 µl at 25 °C with a 3.0-minuterun time. By choosing a shorter column packed with smaller particles, analysis time can be significantly reduced while maintaining column efficiency and resolution. Previously, the smallest commonly used porous particles commonly used were either 3.5 or 3 µm in diameter. In the present study, a C18 column was used as it can easily resolve paracetamol in under 3 minutes with high efficiency.

Method Validation: The proposed UHPLC method has been validated in compliance with the International Conference on Harmonization (ICH) guidelines, encompassing specificity, selectivity, linearity, accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), and robustness [23].

Specificity/ Selectivity: Selectivity refers to the capability of an analytical method to differentiate and measure the analyte in the presence of potential interfering substances in the sample. The selectivity assessment showed no significant response attributable to interfering ingredients at the retention time of the analyte, which was 1.657 min for paracetamol. This method demonstrated high specificity for detecting the paracetamol analyte, despite other components in the formulation.

Linearity and Range: According to the ICH guidelines, the linearity of the analytical method is defined by its ability to obtain results that are directly proportional to the analyte concentration within a given range. Various aliquots of the standard solution from the stock solution were prepared and analyzed to determine the linearity of the used method. The drug showed linearity within the range of 10-150µg/ml with a correlation coefficient (r^2) ≥ 0.997712.

Accuracy and Precision: The intraday accuracy and precision study involved preparing a set of calibration standard curve samples and 12 control samples (QCs) at each concentration [low (25.00 µg/ml), medium (75.00 µg/ml), and high (125.00 µg/ml)] and analyzed within a single day. Additionally, another set of calibration standard curve samples and six QC samples of each concentration (low (25.00 µg/ml), medium (75.00 µg/ml), and high (150.00 µg/ml)) were prepared and analyzed over three days. The mean, % accuracy, SD, and % CV were calculated for both the intraday and interday validation.

Lower Limit of Quantification (LLOQ) and Lower Limit of Detection (LLOD): The LLOD refers to the smallest amount of an analyte detectable in the sample, while the LLOQ is the minimum amount of analyte that can be quantitatively measured with acceptable precision and accuracy. Both LLOQ and LLOD were determined using data from the lowest concentration standard during accuracy and precision runs, as previously described, and by measuring signal/noise ratios (S/N). The mean of these S/N ratios is then utilized to calculate the theoretical LLOD (S/N ≥ 3), and the theoretical LLOQ (S/N ≥ 10).

Application of the Proposed Method for Pharmaceutical Formulation: The assay test for the various strengths of paracetamol tablets was designed to ascertain the precise quantity of the active ingredient. The analysis of each batch involved the preparation of six calibration standards, six QCs, and six assay samples, all of which were intended for injection into the RP-UHPLC method.

RESULTS: This paper outlines the development and validation of a simple, sensitive, precise, and accurate UHPLC method for quantifying paracetamol in pharmaceutical formulations. Several mobile phases and columns were initially tested to achieve an optimal chromatogram. The selected column and the mobile phase were evaluated for selectivity, sensitivity, and acceptable chromatographic parameters of the resulting peaks in terms of sharpness, symmetry, and tailing factor. **Fig. 1** displays a UHPLC chromatogram of varying concentrations of paracetamol from the lowest concentrations (10 µg/ml) to the highest concentrations (150 µg/ml).

The UHPLC chromatography results showed excellent linearity with a determination coefficient (r^2) ≥ 0.997712 and correlation coefficient (r ≥ 0.998844) within the range of 10-150.0µg/ml. The accuracy for both intraday and interday was between 10-110%, and the % CV Error was< 10%. The LLOD was established at 100ng/ml and LLOQ **Fig. 2.**

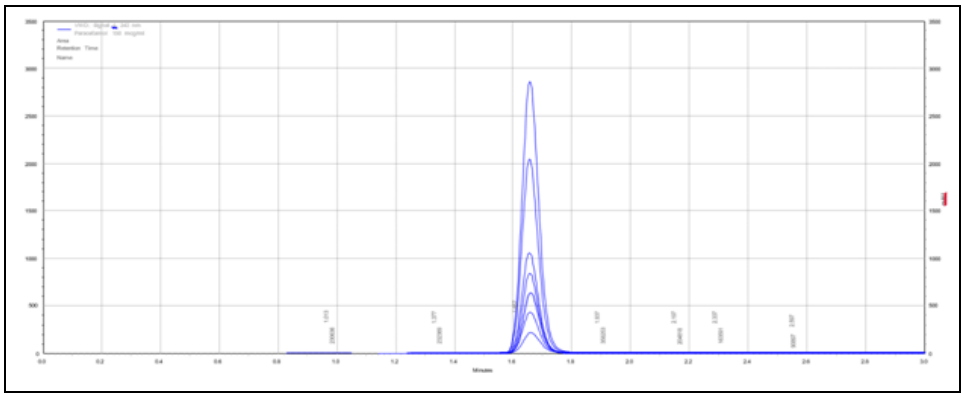

FIG. 1: UHPLC CHROMATOGRAM INDICATING THE LINEARITY OF THE METHOD

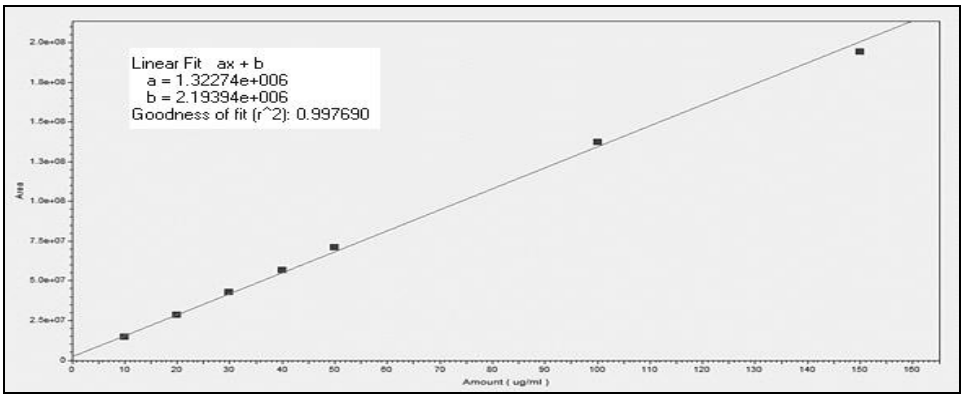

FIG. 2: THE CALIBRATION CURVE OF PARACETAMOL (10 - 150.00 µG/ML) IN WATER

Fig. 2 shows the UHPLC chromatogram indicating the linearity of the method. In addition, the method was applied to a study with high sensitivity. All the validation parameters were performed according to ICH guidelines. The validation data confirmed that the UHPLC method in aqueous solutions is sensitive, accurate, and precise, with the intraday and interday precision assessment (% CV) less than 2% (accepted range ± 10%). The intraday and interday % accuracy of paracetamol ranged from 98%-100% which was in the accepted range of (90-110%), in **Table 1**.

TABLE 1: SUMMARY OF THE ACCURACY AND PRECISION RESULTS OF THE PROPOSED UHPLC METHOD

Proposed methods	Paracetamol Concentration (µg/ml)	Mean	% Accuracy	SD	% CV
Intraday Validation	25.00	25.1018	100.4073	0.1052	0.4190
(Accuracy & Precision)	75.00	76.2811	101.7081	0.1362	0.1785
	125.00	123.7828	99.0262	0.1791	0.1447
Interday Validation	25.00	25.0946	100.3849	0.0830	0.3308
(Accuracy & Precision)	75.00	76.2956	101.7274	0.1603	0.2101
	125.00	123.6797	98.9438	0.1889	0.1527

The chromatographic technique demonstrated that the LLOQ and the LLOD were0.625 µg/ml and 0.156 µg/ml respectively **Fig. 3A, B** & **4**. Three pharmaceutical formulations of paracetamol tablets, Panadol, Panadol (Cold & flu) and Panadol Extra were analyzed by the UHPLC method. The peak area of the sample solution was measured and the amount of paracetamol present in the tablet formulation was determined through the calibration curve. The assay results are summarized in **Table 2**. The percentage content of the active ingredient in the three paracetamol tablets displayed acceptable values of 99.01%-100.83%, which comply with the ICH acceptance criteria of (90%-110%).

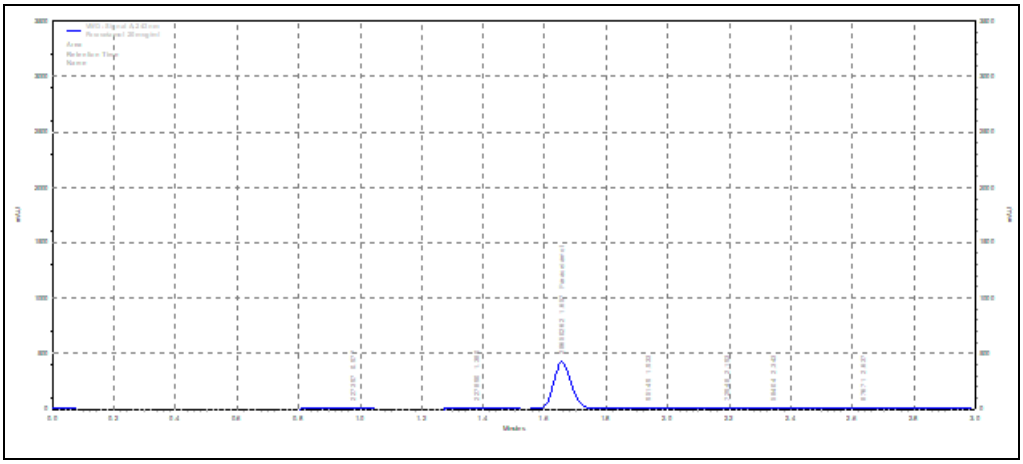

FIG. 3A: UHPLC CHROMATOGRAM OF 30 MG/ML SELECTED FROM CALIBRATION CURVE OF VARYING CONCENTRATIONS OF PARACETAMOL

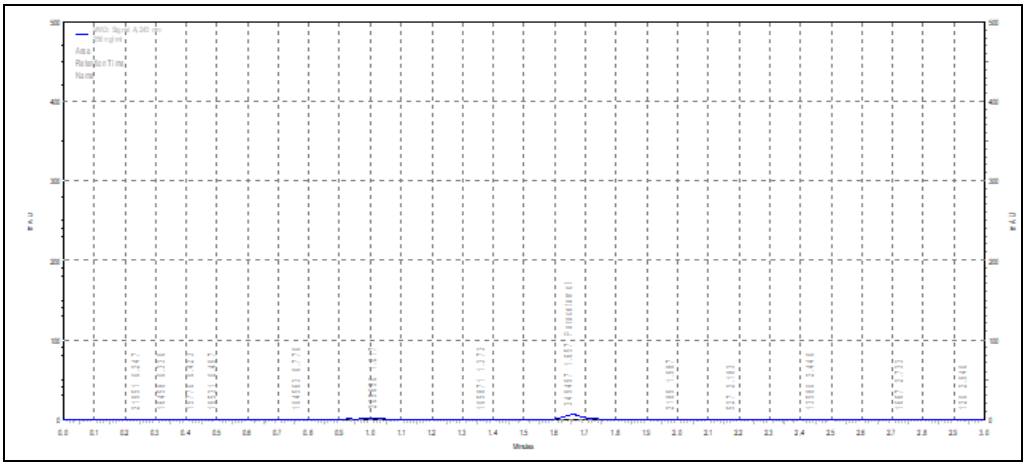

FIG. 3B: THE LOWER LIMIT OF QUANTIFICATION (LLOQ) OF PARACETAMOL WITH THE SAME Y AXIS

TABLE 2: PARACETAMOL ASSAY TESTS WERE ANALYZED BY THE UHPLC

S. no.	Name of Product	Strength (mg)	Test product concentration (µg/ml)	Average (of six samples)	%Assay	STDV	%RSD
1	Panadol	500	100	99.0157	99.0157	0.12165	0.12286
2	Panadol (Cold+ flu)	500	100	100.8360	100.8360	0.11545	0.11449
3	Panadol Extra	500	100	99.54483	99.54483	0.13107	0.13167

The chromatogram showed the separation of paracetamol (500 mg tablets) Panadol, Panadol (Cold+ flu) and Panadol Extra both made to an injectable dilution of 100µg/ml in **Fig. 5, 6** & **7**.

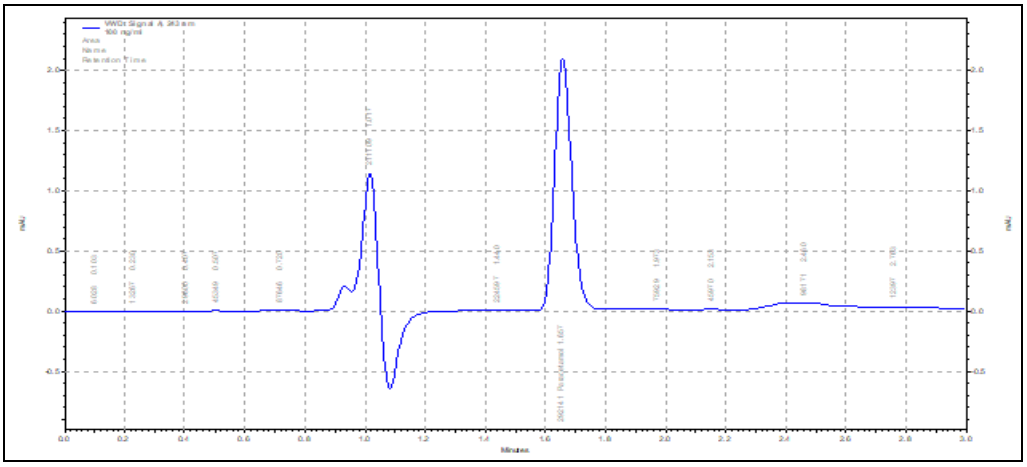

FIG. 4: THE LOWER LIMIT OF DETECTION (LLOD) OF PARACETAMOL

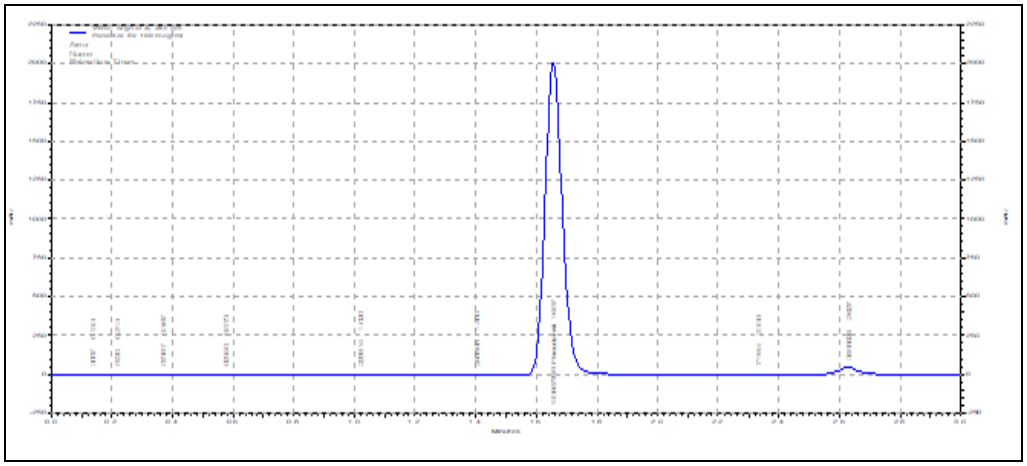

FIG. 5: THE CHROMATOGRAM OF PANADOL EXTRA 500 MG TABLETS

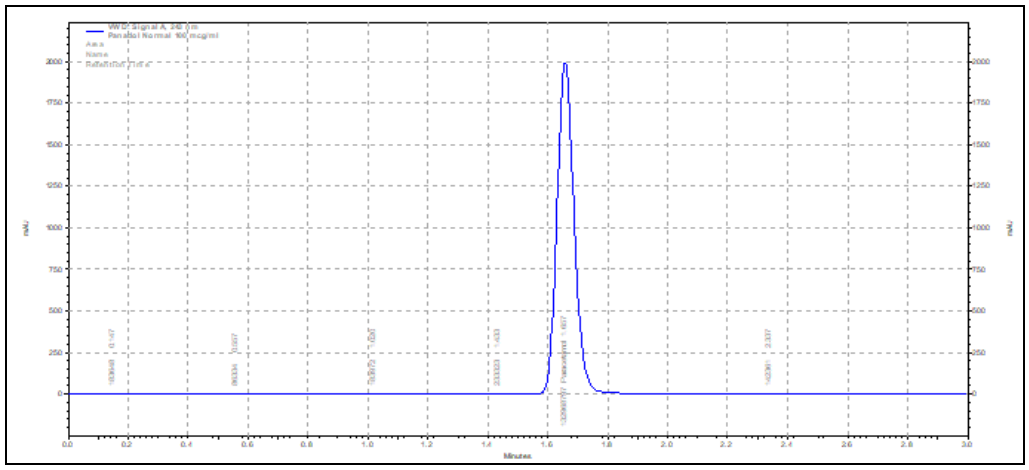

FIG. 6: THE CHROMATOGRAM OF PANADOL 500 MG TABLETS

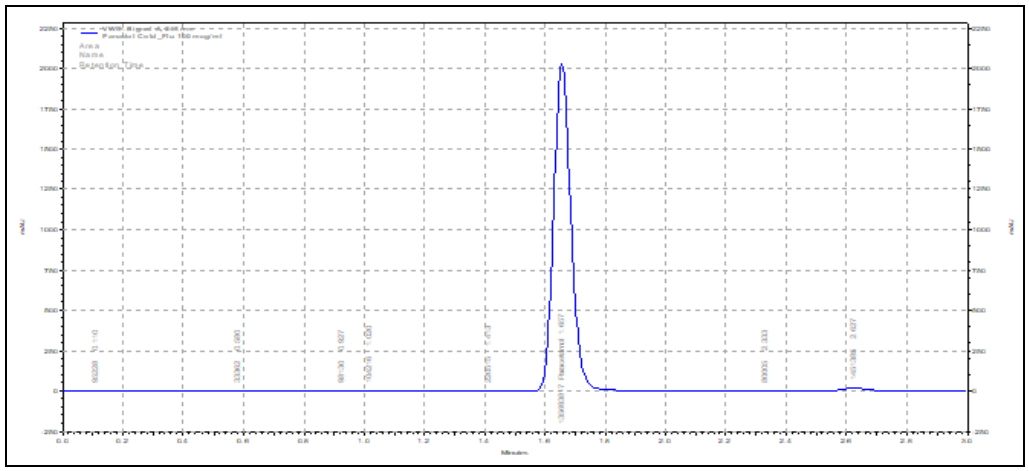

FIG. 7: THE CHROMATOGRAM OF PANADOL COLD AND FLU 500 MG TABLETS

DISCUSSION: The results also demonstrated high sensitivity, allowing for drug detection at the nano-gram level and recoveries between 99.01%-100.83%, which confirmed the effectiveness of the UHPLC method in quantifying paracetamol in different pharmaceutical formulations. The validated analytical techniques in the present study ensured accuracy, precision, linearity, and sensitivity compared with the previously reported HPLC methods. The present study was carried out using different analysis methods that were validated according to ICH guidelines. The validated analytical methods enable accurate and sensitive quantification of paracetamol. The UHPLC method was more accurate, sensitive, precise, linear, and. These analytical methods are

applicable in any in-vitro experiments such as quality control and stability tests.

CONCLUSION: This study highlights the value of UHPLC analysis using affordable and readily available solvents commonly found in any quality control laboratory. This rapid method with a 3 min runtime, combined with reduced solvent consumption, offers substantial economic benefits.

ACKNOWLEDGEMENT: We express our gratitude and appreciation for the help and support received in the process of this research article finalization. I also extend my heartfelt thanks to KAIMRC and department of drug analysis for their unwavering support.

Data Availability Statement: Data will be made available on request.

Credit Authorship Contribution Statement: Badran Alyahya, Abdullatif Al-Dulaimi and Wesam Abdel-Razaq: contributed to the methodology and writing the manuscript. Ibrahim Farh, Waleed Alhussaini, Amani Kurdi: Supervised all practical work, and reviewed the manuscript. Abdulkareem AlBekairy, Imadul Islam, Sultan Alqahtani and Mahmoud Mansour: Suggested the research point and experimental design.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the King Abdullah International Research Center, National Guard Health Affairs, Riyadh, Saudi Arabia (grant no. SP22R/218/09). The funding agency had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Mansour MA, Alyahya B, Al-Dulaimi A, Farh I, Alhussaini W, Kurdi A, Alqahtani S, Abdel-Razaq WS, Islam I and Albekairy AM: Comparative performance of liquid chromatography and spectrophotometry for quantitative analysis of paracetamol in tablets formulations. Int J Pharm Sci & Res 2026; 17(3): 1025-32. doi: 10.13040/IJPSR.0975-8232.17(3).1025-32.