Iranian Journal of Pharmaceutical Sciences
2025: 21 (1): 32- 47
https://journals.sbmu.ac.ir/IJPS

Research Article

Pharmacokinetics Study of 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acidusing spectrophotometric method

Pravin Patil[a*], Khanderao Pagar[a], Pramod Patil[b]

[a] *Department of Chemistry, SSR College of Arts, Commerce and Science, Silvassa, Sayli Road, DNH, 396230, India.*
[b] *Tarsadia Institute of Chemical Sciences, Uka Tarsadia University, Bardoli, Gujarat-394350, India.*

Received: June 8, 2024 **Last Revision:** October 24, 2024 **Accepted:** November 8, 2024 **Available online:** February 1, 2025

Abstract

A spectrophotometric method was derived to determine 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid (PPQC). This analytical method was simple, sensitive and low-cost. Based on forming of a chelate complex between PPQC and Fe(III), the developed method produced a yellow-colored complex that was detectable at 418 nm at room temperature. As per the International Conference on Harmonization guidelines, several analytical parameters for the suggested method were verified. With a correlation coefficient of 0.9998 (n=6) and a molar extinction coefficient of 1.2967×10^4 L mol^{-1} cm^{-1}, the method was linear from 5 to 46 µg mL^{-1}. The limit of detection (LOD) and limit of quantitation (LOQ) for the described method were 0.69 µg mL^{-1} and 2.09 µg mL^{-1}, respectively. It was carefully examined and optimized how different parameters affected the chelate complex reaction. The recoveries of PPQC from spiked blood samples were 98.40-100.40%. The developed method's coefficient of variation (CV) was less than 2.07%. The method validation was determined in accuracy, precision, absolute recovery, freeze–thaw stability, bench-top stability and long-term stability. After oral administration of PPQC to Wistar albino rats, the method's analytical recovery, sensitivity, and accuracy were adequate to characterize the pharmacokinetics study. Thus, pharmacokinetics data for pre-clinical research have been obtained efficiently through the assay method.

Keywords: Anhydrous Ferric Sulfate; Biological fluid; 1-ethyl-6-fluoro-1; 4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid; Pharmacokinetics study; Spectrophotometric method.

1. Introduction

Over the past 50 years, people have used paracetamol (PCM) (N-acetyl-p-aminophenol) drug, as an analgesic and antipyretic [1]. It is frequently used to treat fever and pain in both adults and children [2]. The pharmacological properties of paracetamol and its many derivatives are also widely known [3-7]. PPQC **(Figure 1)** is one of the recently synthesized paracetamol derivatives [3]. PPQC has been synthesized by combining (4-Amino N-acetyl) phenyl 2-chloro acetate and 1-Ethyl-6-fluoro-1, 4-

*** Corresponding Author**:
Pravin Patil, Department of Chemistry, SSR College of Arts, Commerce and Science, Silvassa, Sayli Road, DNH, 396230, India.
E-mail: bholenath1983@rediffmail.com.

Cite this article as: Patil P., Pagar K., Patil P., Pharmacokinetics Study of 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acidusing spectrophotometric method., Iran. J. Pharm. Sci., 2025, 21 (1): 32- 47.

DOI: https://doi.org/10.22037/ijps.v21i1.45493

dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid using solvent DMF and $NaHCO_3$ [3]. It has been shown that the pharmacologically active molecule PPQC exhibits analgesic, antipyretic, anti-inflammatory, and antibacterial properties [3].

Various advanced methods for identifying paracetamol alone or in combination with other medications are available in the literature. These methods include high-performance thin-layer chromatography (HPTLC) [8, 9], gas chromatograph mass spectrometry (GC–MS) [10], high-performance thin-layer chromatography (HPLC–UV) [10–12], high-performance thin-layer chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) [13], and micellar electrokinetic capillary chromatography (MECC) [14]. The many applications, robustness, simplicity, and ease of use of UV spectrophotometer methods make them preferred over other analytical techniques. Additionally, employing UV spectrophotometers, analysis can be done when necessary in locations other than the main laboratory. Because of this, such a technique may be created to calculate the PPQC in biological samples for pharmacokinetic research.

Various methods have been used in literature to demonstrate the binding of ions or molecules with certain metal ions to form a chelate complex [15-19].After a review of the literature, it was also found that polydentate (multiple bonded) ligands interact with metal through two or more co-ordination bonds, demonstrating their attraction for metal ions [20, 21].Bhardwaj N et al. [20] developed a spectrophotometric technique based on the measurement of color complexes formed by Cu(ll), Zn(ll), and Cd(ll) with labetalol using an aqueous buffer solution at 350 nm. While examining complex stability, the strategy was successfully optimized. Rajendraprasad N et al. [21] developed two spectrophotometric methods for the direct estimation of iron (III) in pure form, leaves, and pharmaceuticals by employing salicylic acid (SCA) as a chromogenic agent.

The developed method generated chromogens with maximum absorption at 520 and 460 nm, respectively, using a complexation reaction between iron (III) and SCA at pH 2.26 (±0.02) for Method A and 6.1 (±0.02) for Method B. Using a 0.0001 M concentration of Al (III) ion and a pH range of 4.5-6.0, Al-Nuzal et al.[22] developed the aluminum(III)-tetracycline complex formation in drug formulation, which was measured at 370 nm. A spectrophotometric technique was developed to evaluate the strategy, and statistics were employed by El-Bagary R. I. et al. [23] to identify the presence of rivaroxaban and cilostazol in drug formulations.

The methodology involved the oxidation of the selected medications by iron (III) in the presence of 1, 10-phenanthroline, leading to the formation of the tris (1, 10-phenanthroline) iron (II) complex (ferroin), with a λmax at 510 ± 1 nm. The recovery and analytical parameters analyzed were reported. A spectrophotometric method was developed by Azmi S. N. H et al. [24] to quantify piroxicam in pharmaceutical dosage forms. The process was based on the drug's chelation with Fe(III) to form a pink metal chelate with maximum light absorption at 504 nm at room temperature.

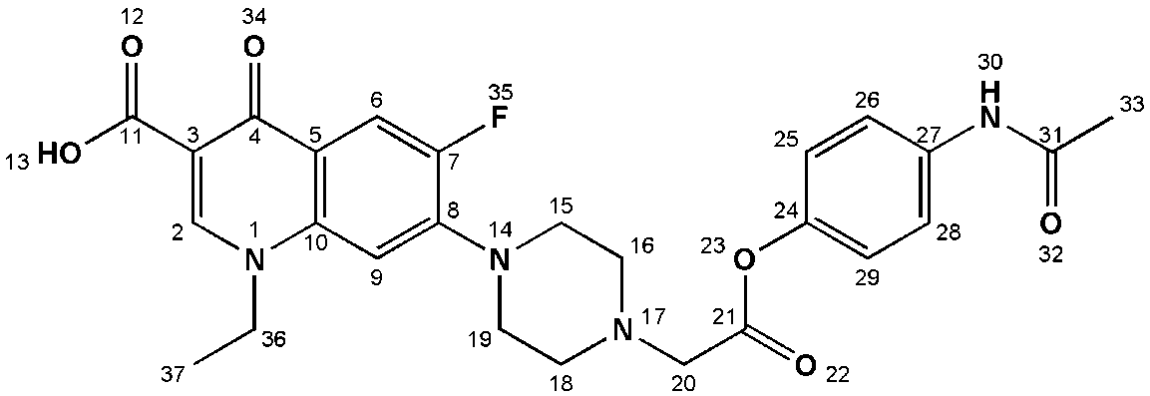


Figure 1. Chemical structure of 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid (PPQC).

The quantitation and detection limits obtained with the recommended method were 2.348 μg mL^{-1} and 0.775 μg mL^{-1}, respectively. Dosage forms were treated using the method with good outcomes. A spectrophotometric technique based on chelating ferric ions with piroxicam to create a colored metal complex at room temperature was described by Lutfullah et al. [25]. The complex was measured at 495 nm. The procedure was evaluated using statistics. Two spectrophotometric techniques have been published by Al-Hammoodi I. A. S. et al. [26]. These techniques involve oxidizing terbutaline sulfate with Fe(III) in a nitric acid medium, followed by Fe(II) chelation with 1,10-phenanthroline (1,10-Phen) in method A and 2,2´-bipyridyl (2,2'-bipy) in method B. The measurements were taken at 522 and 510 nm, respectively.

A statistical analysis of the processes was conducted. This method was successfully used to determine the amount of terbutaline sulfate in its commercial formulations as a syrup and tablet. A technique for measuring the sodium of omeprazole (OMZ) and pantoprazole (PNZ) based on the creation of 2:1 chelates of both drugs with different metal ions was described by Salama F. et al. [27]. Pantoprazole sodium was determined by chelating iron (III) in an aqueous-ethanol medium to produce an orange chelate, measured at 455 nm, using a stability-indicating technique. The colored chelates of OMZ in ethanol were detected spectrophotometrically at 411, 339, and 523 nm, respectively, using iron (III), chromium (III), and cobalt (II). The process was validated using statistics. Gaber M. et al. [28] developed a method for spectrophotometric estimation of norfloxacin by measuring the drug in dose and pure forms. The compounds were measured at 425 and 415 nm, forming colored complexes with Fe (III) and Cu(II). A statistical analysis of the processes was done using aqueous buffer solutions.

PPQC is a newly developed paracetamol derivative. A suitable and sensitive analytical method must be developed to estimate PPQC at low serum or other biological fluid sample levels. There are no official methods for determining PPQC. It was the first method for determining PPQC in spiked blood samples, and it is also used to evaluate pharmacokinetics parameters.

As far as we know, no published spectrophotometric analytical method for evaluating PPQC in biological fluid exists. The developed technique is a simple, quick, low-cost way to measure PPQC in biological fluid using spectrophotometry. Based on forming of a chelate complex between PPQC and Fe(III), the proposed method yields a yellow product with a maximum absorbance of 418 nm. The suggested process is quick, uses little solvent volume and less expensive solvents, and is economical. Additionally, this technique does not require highly sophisticated instruments (like, HPLC and GC). The developed spectrophotometric approach for PPQC assessment in biological fluids has been effectively implemented. The established technique was also applied to assess the pharmacokinetics research of PPQC in Wistar albino rats following oral administration (600 mg Kg^{-1} body weight). The Institution Animal Ethical Committee authorized all procedures for animal experimentation.

2. Materials and Methods

2.1. Chemicals

The synthesized compound; PPQC was used after recrystallization and purification. The following materials were acquired from Sigma-Aldrich in Mumbai, India: Anhydrous Ferric sulfate ($Fe_2(SO_4)_3$), Potassium ferricyanide [$K_3Fe(CN)_6$], Sulphuric acid, Hydrochloric acid, Ethyl acetate and Methanol.A gift sample of paracetamol (Purity > 99.9) was obtained from Cadila Pharmaceutics, in Ahmedabad, Gujarat, India. Additional analytical-grade chemicals and double-distilled water were employed during the experiment.

2.2. *Animals*

For this investigation, Wistar albino rats weighing 150–500 g were utilized. They were used for collecting blank blood samples and were maintained at -20 °C. Institutional Animal Ethical Committee (VBT/IAEC/10/12/40) and Vidyabharti Trust College of Pharmacy, Umrakh, Bardoli, Gujarat, India, approved this study.

2.3. Apparatus

A double beam, UV-visible spectrophotometer (1700-Shimadzu Precision Instruments, Japan) with a quartz cell and a 1.0 cm path length was used to perform the spectrophotometric determination of PPQC. Analytical

balance with a minimum weight capacity of 10 mg and a maximum of 220g, AUW220D, Shimadzu, Japan, was used. A cooling centrifuge (C-24BL, maximum rpm 20,000, minimum cooling temperature -8 ˚C, Remi Equipment Pvt. Ltd., Mumbai, India) was used to prepare of blood samples.

2.4. Preparation of Stock Solutions

An aqueous stock solution of 0.5 M $H_2SO_4$was created in a 100 mL volumetric flask by dissolving 2.8 mL of H_2SO_4 in 100 mL of double-distilled water. Weighing 0.19 g of anhydrous $Fe_2(SO_4)_3$and combining it with 1.0 mL of 0.5 M H_2SO_4 in a 100 mL volumetric flask resulted in an acidic stock solution of 5.0 mM $Fe_2(SO_4)_3$. The volume was adjusted to the appropriate level using double-distilled water to achieve the final concentration of 5.0 mM $Fe_2(SO_4)_3$.

The PPQC was precisely weighed (0.1 g) and dissolved in the appropriate solvents in a 100 mL volumetric flask to create the aqueous (stock solution-1) and methanolic (stock solution-2) stock solutions of PPQC (1.0 mg mL^{-1}). Various working solutions were prepared by adapting the simple dilution method.

After dissolving 100 mg of paracetamol in 100 mL of double-distilled water, a standard solution of 100 µg mL^{-1} paracetamol (stock solution-3) was created. It was then further diluted as needed. Weigh a sample of potassium ferricyanide precisely to weigh 0.03 g, then dissolve it in 100 mL of double-distilled water to create a stock solution of 1.0 mM potassium ferricyanide. 0.04 g of anhydrous $Fe_2(SO_4)_3$ was added to a 100 mL volumetric flask and diluted with double-distilled water until the required concentration was obtained to create a 1.0 mM stock solution of $Fe_2(SO_4)_3$. To make a stock solution of 1.0 M HCl, 8.8 mL of concentrated HCl was transferred to a 100 mL volumetric flask and diluted with double-distilled water to the appropriate level.

2.5. Stock Solutions of Interfering Radicals

The following interfering substances were dissolved in the appropriate volumes of double-distilled water to create 100.0 µg mL^{-1} solutions: Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Glycine, Tyrosine, L-Alanine, glucose, uric acid, L-ascorbic acid, and paracetamol. A dilution technique was used to create the necessary concentrations during the study.

2.6. Determination of Wavelength (λ_{max})

1.0 mL of standard solution-1 of PPQC (1.0 mg mL-1) was added to a 10 mL volumetric flask. It was then combined with 1.0 mL of $Fe_2(SO_4)_3$stock solution and diluted with double-distilled water to get the required concentration. The final product had an intensely colored complex, which was then scanned using a UV-visible spectrophotometer in the 400-650 nm range, and the maximum wavelength was measured against a blank reagent.

2.7. Optimization Studies

2.7.1. Effect of Diluting Solvent

The effect of diluting solvent was investigated using various solvents, including acetonitrile, ethanol, methanol, double-distilled water, propanol, and butanol. The effect of diluting solvent was measured using 10.0 µg mL^{-1} of PPQC solution. A certain amount of PPQC (from stock solution-1) was taken in a 10 mL volumetric flask and thoroughly mixed after adding 1.0 mL of acidic $Fe_2(SO_4)_3$stock solution. Ultimately, acetonitrile, ethanol, methanol, double-distilled water, propanol, and butanol were solvents to dilute the resultant solution to the appropriate level. The absorbance was measured at λmax using the appropriate blank solvents.

2.7.2. Effect of Reagent Concentration

The effect of concentrations of H_2SO_4 and $Fe_2(SO_4)_3$were found to have an impact on absorbance in the ranges of 0.1-2.5 M and 0.1-5.0 mM, respectively. A constant volume of PPQC (10.0 µg mL-1) was used for this investigation. Each 10 mL volumetric flask contained an appropriate aliquot of PPQC taken from the stock solution-1. After that, 2.0 mL of $Fe_2(SO_4)_3$stock solutions were added to each flask, which were made with different concentrations of H_2SO_4. Double-distilled water was used to dilute the final solutions to the appropriate amount. Comparably, various $Fe_2(SO_4)_3$ concentrations and a fixed quantity of H_2SO_4 were studied. The optimal concentration of reagents was found at 418 nm against the blank.

2.7.3. Effect of Reaction Temperature and Time

The reaction temperature and time were optimized by employing the steepest ascent technique [29]. The stock solution-1 (10.0µg mL^{-1}) of PPQCwas used to optimize

reaction temperature and time studies for PPQC. According to the spectrophotometric method of PPQC, an appropriate aliquot of PPQC from the stock solution-1 was mixed with reagents in a vortex mixer. Then, it was incubated at various temperatures, ranging from 5 to 60 ºC.Absorbance was measured using λmax at different temperatures. Each determination was done in triplicate. The optimal reaction time for PPQC was determined based on the effect of temperature. The absorbance of the resulting solutions was measured at various time intervals ranging from 0 to 30 minutes. At the optimal reaction temperature, when the absorbance of the sample reached its maximum, that was considered the optimal reaction time.

2.7.4. Stoichiometric Ratio Determination

The stoichiometric ratio between the synthesized compound PPQC and reagents was tested with the Job method [30]. The stoichiometric ratio was determined using the equimolar (1.0 mg mL^{-1}) solutions of PPQC and $Fe_2(SO_4)_3$. Seven distinct $Fe_2(SO_4)_3$ volumes, 0.00, 0.4, 0.66, 1.00, 1.34, 1.60, and 2.00, were collected into 10 mL volumetric flasks and subsequently diluted to a volume of 2.0 mL using PPQC solution. The final step involved diluting the resultant solutions to 10.0 mL using double-distilled water. At 418 nm, the absorbance was measured about the blank.

2.8. Method Validation

The primary criteria for evaluating the spectrophotometric approach were the linearity assay, accuracy, and precision. This method was developed and validated by ICH guideline 2023 [31].

2.8.1. Linearity

Six dilutions, ranging from 5 to 30 µg $mL^{-1}$and 4 to 14 µg mL^{-1}, were made from stock solutions 1 and 2. A paracetamol calibration curve was created using the stock solution-3, ranging from 0.2-2 µg mL^{-1}. A calibration curve was created to obtain the linearity and regression equations after the absorbances of each resultant solution were measured at λmax of paracetamol (700 nm).

2.8.2. Precision and Accuracy

The precision and accuracy of the developed method were assessed at three spiked concentrations of PPQC in blood samples (1.25, 2.50, and 3.75 µg mL^{-1}) [29]. This investigation used spiked PPQC samples during intra- and inter-day analysis. The precision of the developed method was described using the coefficient of variation (CV). As a percentage of the measured PPQC concentration, the accuracy was stated. Each blood sample was examined three times on the same day to evaluate the intra-day precision and accuracy of the developed method. On the other hand, each sample was analyzed for six days to evaluate the inter-day accuracy and precision of the developed method.

2.8.3. Recovery Study

The recovery study was carried out using three distinct spiked concentrations of PPQC in blood samples (1.25, 2.50, and 3.75 µg mL^{-1}). The recovery study was expressed as a percentage of the PPQC-measured concentration.

2.8.4. *Robustness*

The impact of small variations in the procedure was examined to evaluate the robustness. Some parameters were changed in these experiments, but others remained the same. The calculations were done using standard deviation and CV. The robustness of the proposed method was assessed using changes in the analytical wavelength (±1 nm), working temperature (±2 ºC), and reaction time (±3 min) at two different concentrations (5.0 and 10.0 µg mL^{-1}) analyte.

2.8.5. Ruggedness

To assess the robustness of the proposed methods, two analysts each used two different analyte concentrations (5.0 and 10.0 µg mL^{-1}).Day-to-day reproducibility was studied over six consecutive days. Ruggedness was found in terms of standard deviation and CV.

2.8.6. Stability Study

The stability study of spiked PPQC in blood samples was measured under typical handling and storage conditions. Spiked blood samples (1.25, 2.50, and 3.75 µg mL^{-1}) were kept under freeze-thaw conditions, bench top conditions for 6 to 24 hrs at room temperature, and long-term conditions for 5 weeks at -20 ºC. The freeze-thaw stability of the studied drug was determined over three freeze-thaw cycles within 3 days. In each cycle, the

frozen plasma samples were kept at room temperature for two hours and refrozen for 24 hours. After completion of each cycle of the freeze-thaw, bench-top and long-term samples were analyzed by comparing them with newly prepared intra-day spiked blood samples using the PPQC assay method that was developed.

2.8.7. Specificity/Selectivity

The interferences of other possible substances on the PPQC determination were used to assess the specificity and selectivity of the method. The developed method was used to assess the effect of interfering species (like Ca^{2+}, Na^{+}, K^{+}, Mg^{+2}, Zn^{+2}, Fe^{+2}, L-Alanine, Glycine, Tyrosine, glucose, uric acid, paracetamol and L-ascorbic acid) added to spiked blood samples containing a fixed amount of PPQC (10.0 µg mL^{-1}). The recommended approach's selectivity and tolerance to different interferences were assessed.

2.9. Determination of PPQC in BiologicalFluid

The published procedure was employed to determine PPQC in blood samples that had been spiked [32, 33]. A 0.5 mL sample of blank blood was taken from an adult Wistar albino rat through a retro-orbital sinus puncture, and the sample was then put into a 2 mL polyethylene centrifuge tube. This 0.5 mL blood sample was spiked with a 0.5 mL stock solution-2 of PPQC (1.0 mg mL^{-1}), and it was then added to a centrifuge tube that had been previously filled with 1.0 mL of 1:2 mixture of methanol and ethyl acetate solvents. The mixture was thoroughly mixed after five minutes of manual shaking. A literature survey [32, 33] revealed that a mixture of 1:2 methanol and ethyl acetate was the best solvent for blood extraction because neither methanol nor ethyl acetate alone could precipitate blood proteins. After being agitated on a vortex mixture for five minutes, the resultant mixture was centrifuged for five minutes at 2000 rpm at room temperature.The clear supernatant was collected and did not need any more clean up. The proper volumes of the supernatant were put into 10 mL volumetric flasks and evaluated using the devised procedure. Using the suggested approach, the relevant concentrations of PPQC (1.25, 2.50, and 3.75 g mL^{-1}) in blood supernatants were examined at maximum wave length against the blank reagent. Except for adding PPQC, the blank reagent was made similarly. The calibration curve for spiked PPQC in blood samples within the range of µg mL^{-1} was generated.

2.10. Pharmacokinetics Study

2.10.1. Administration of PPQC

The animals were split into two groups, with four rats in each group. The synthesizedPPQCcompound and paracetamol dosages were produced in an aqueous 2.0% acacia gum solution. Paracetamol (500 mg kg^{-1}) was administered to the animals in group I as a reference standard and control. Group II animals received 600 mg kg^{-1} of PPQC orally.

2.10.2. Pharmacokinetics Analysis

The pharmacokinetics of the synthesized compound PPQC was studied using the non-compartment animal model and the Trapezoidal rule [34, 35]. A pharmacokinetics investigation was conducted using The AUC_{0-t} and $AUC_{0-\infty}$ values were computed using the drug concentration versus time curve. The formula for the AUC0-t and AUC0-∞ is calculated as:

$$AUC_{0-t} = \sum_{i=0}^{n-1} \frac{(t_{i+1} - t_i)}{2} (C_i + C_{i+1})$$

and

$$AUC_{0-\infty} = AUC_{0-t} + \frac{Cp\ last}{K'}$$

The semi-log curve of the drug's concentration versus time t yields the concentration (Cp last) of paracetamol and PPQC at time t and the constant (K' = slope). The area under the first moment curve, or $AUMC_{0-\infty}$ values, are computed using the drug concentration and time vs. time t curve [$AUMC_{0-\infty}$= $AUMC_{0-t}$ + $AUMC_{t-\infty}$]. The concentration of PPQC and paracetamol at time t is found using the semi-log curve of drug concentration and time (Cp*T last) vs. time. The AUC value indicates the amount of drug absorption. The mean residence time, or MRT, is the average time drug molecules stay in the body from a given dose [MRT = $AUMC_{0-\infty}/AUC_{0-\infty}$]. The half-life study indicates the duration required for a drug's concentration to drop to half of its initial value [$t_{1/2}$ = 0.693/Kel]. The volume into which a drug appears to be distributed with a concentration equal to that of plasma. The apparent volume distribution at equilibrium is the volume into which a drug appears to be distributed with a concentration equal to that of plasma. Bioavailability

refers to the rate and extent to which the active ingredient is absorbed from a drug product and becomes available at the site of action. The relative bioavailability is, F = $[AUC_{0-\infty}]_{Test}$ / $[AUC_{0-\infty}]_{Ref}$. The apparent volume distribution at equilibrium is calculated using Vss/F = Dose × $AUC_{0-\infty}$ / $AUMC_{0-\infty}$, where, F= Oral drug availability.

2.10.3. Collection of Blood Samples

From the Wistar albino rat's tail vein, blood samples (around 1.0 mL) were taken and placed in Eppendorf test tubes [3]. A 1:2 methanol and ethyl acetate mixture was first added to an Eppendorf test tube. Blood was taken at 0.0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 10, 12, and 24 hours after the rat was given the PPQC (600 mg kg^{-1}). Then after, a blood sample was immediately added to the 1:2 ratio of methanol and ethyl acetate, and the liquid was manually agitated for five minutes to ensure appropriate mixing. The tubes were then shaken on a vertex mixture for five minutes and centrifuged at room temperature at 2000 rpm. Blood supernatants were separated, labeled, and kept at -20 °C until analysis.

2.10.4. Assay Method for Paracetamol

The reported spectrophotometric method determined paracetamol levels in blood samples [1]. After treatment, 0.2 mL of the blood supernatant sample was mixed with 1.0 mL of 1.0 M hydrochloric acid and 2.0 mL of 1.0 mM ferric sulfate. The resulting solution was placed in a water bath and heated to 100 °C for ten minutes. Subsequently, 2.0 mL of 1.0 mM potassium ferricyanide was added, and 10 mL was diluted with double-distilled water. The final samples were analyzed spectrophotometrically at λmax 700 nm after 24 minutes. The mean value ± standard deviation (SD) (n = 3) was computed for the pharmacokinetics parameters. Students' t-tests and one-way ANOVA were used for statistical interpretation. The threshold for statistical significance was set at $P < 0.05$.

3. Results and Discussion

The chelate complex formation between ferric ions (Fe^{3+}) and PPQC served as the basis for the developed method. **Figure 2** shows that the ester group in PPQC was tautomerized into the enol form and formed a coordination bond with the Fe^{3+} ion. At 40 ºC, a yellow-colored chelate complex was formed due to the interaction between the enol form of PPQC and the Fe^{3+} ion. Here, the stoichiometry of the complex formation was the one mole of the Fe^{3+} ion bound with three moles of PPQC in its enol form. The absorbance of the chelate complex was measured at 418 nm concerning a blank reagent to calculate the PPQC concentration based on the absorbance readings using a spectrophotometer. **Scheme 1** depicts the mechanism for the formation of the Fe-PPQC chelate complex.

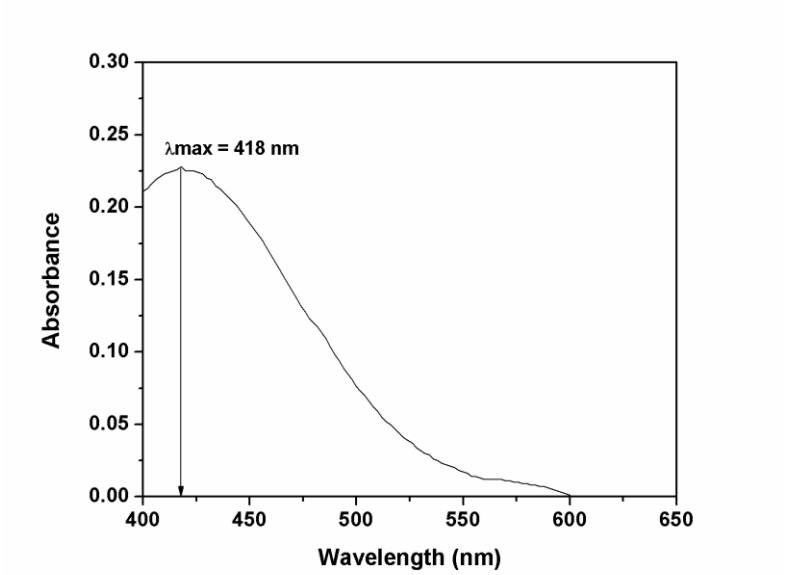
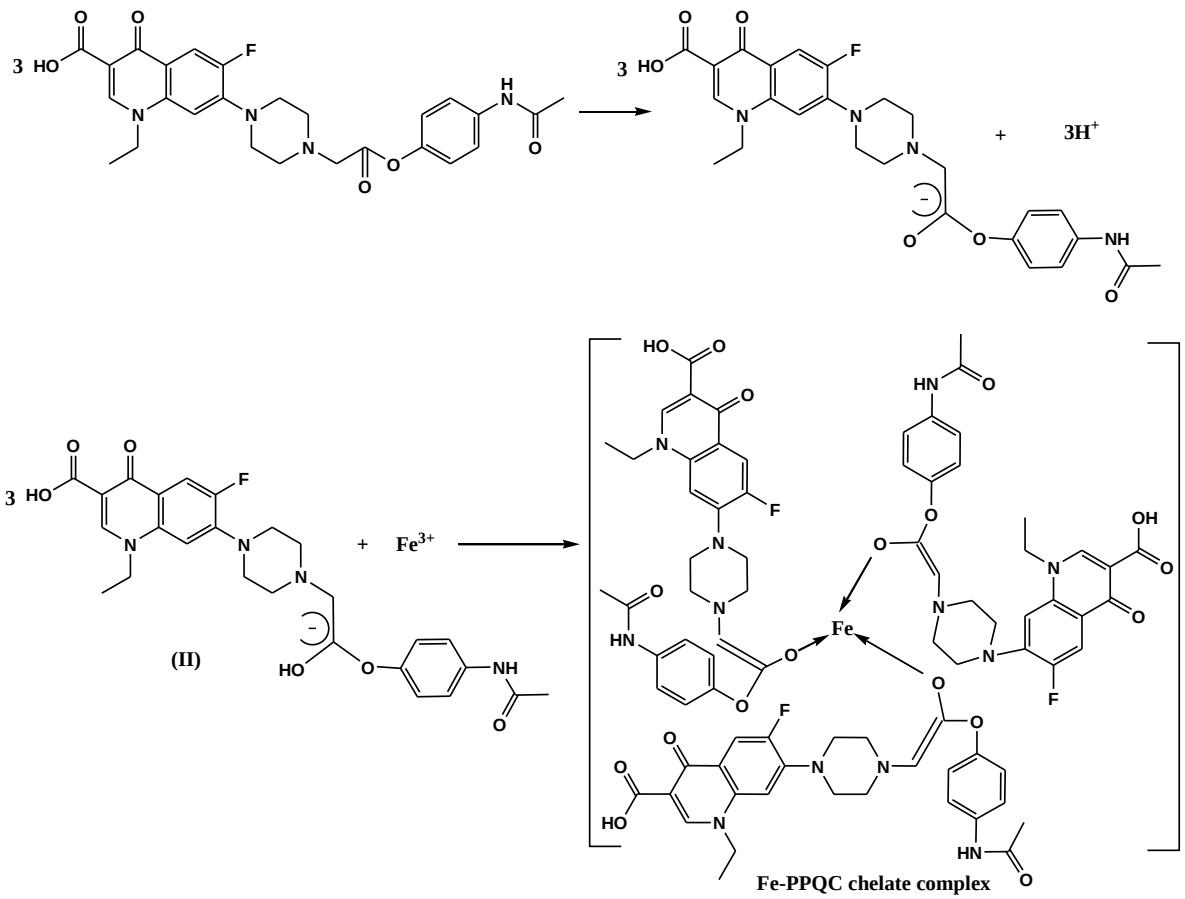


Figure 2. Absorption spectra of the yellow-colored Fe-PPQC chelate complex(1.0 mg mL^{-1} concentration of PPQC) in double distilled water obtained through scanning at various wavelengths. The maximum wavelength (λ_{max}) was 418 nm.



Scheme 1. Reaction scheme for forming PPQC-$Fe_2(SO_4)_3$ chelate complex between PPQC and $Fe_2(SO_4)_3$.

3.1. Optimization studies

3.1.1.Effect of Diluting Solvents

The developed spectrophotometric method for PPQC was repeated with various solvents, including acetonitrile, methanol, ethanol, propanol, butanol, and double-distilled water, at fixed concentrations of 10.0 µg mL^{-1}. **Table 1** displays the effect of diluting solvents. The outcomes demonstrated that, in ideal circumstances, the water solvent had the maximum absorbance at 418 nm. Water is a more polar solvent than the diluting solvents. In this solvent, easy ionization occurs, and stable Fe-PPQC chelate complex forms. Therefore, water was used in the developed method as a dilution solvent.

3.1.2. Effect of Reagent Concentration

A fixed volume (10.0 µg mL^{-1}) of PPQC and different molar concentrations of H_2SO_4 and $Fe_2(SO_4)_3$ were added. Absorbance was measured using the developed spectrophotometric method. According to **Scheme 1**, Fe^{3+} ions can quickly form a co-ordination bond with the enol form of the PPQC in an acidic medium, resulting in a stable Fe-PPQC complex. According to the results shown in **Figure 3**, 0.5 M H_2SO_4 and 4.0 mM $Fe_2(SO_4)_3$ were found to be appropriate reagent concentrations for the suggested method.When the experiment was carried out at concentrations below or above 0.5 MH_2SO_4 and 4.0 mM $Fe_2(SO_4)_3$, it was found that it was less effective in the formation of stable Fe-PPQC chelate complex.

Table 1. The effect of diluting solvents on Fe-PPQC chelate complex absorption at 418 nm.

Sr. No	Solvents	Absorbance
1.	Acetonitrile	0.442
2.	Methanol	0.306
3.	Ethanol	0.184
4.	Propanol	0.148
5.	Butanol	0.101
6.	Water	0.447

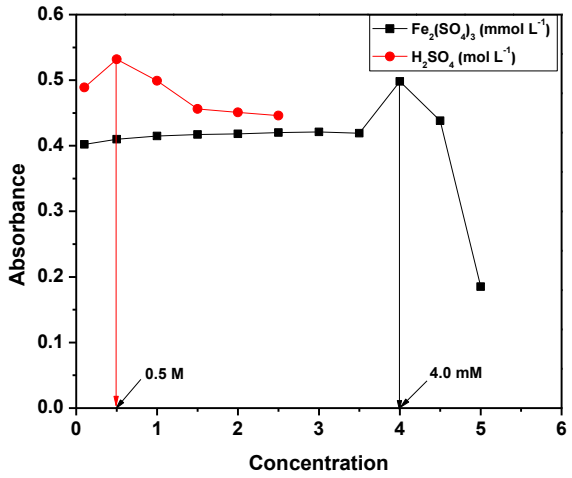


Figure 3. Effect of reagent concentration on formation of Fe-PPQC chelate complexusing (10.0 µg mL^{-1}) PPQC, and various concentrations of H_2SO_4 and $Fe_2(SO_4)_3$ reagents.

3.1.3. Effect of Reaction Temperature and Time

The reaction temperature and time were optimized by employing the steepest ascent technique [29]. The effects of temperature on the absorbance of a (10.0 µg mL^{-1}) PPQC solution at 418 nm was examined over a temperature range of 5-60 ºC, with a five degree celsius difference between the two observations **(Figure 4)**. The results showed that between the lower (5–30 ºC) and higher (45–60 ºC) temperature ranges; there was no apparent difference in absorbance. The experiment revealed that the absorbance was highest at 40 ºC; consequently, this temperature was optimal for the recommended procedure.

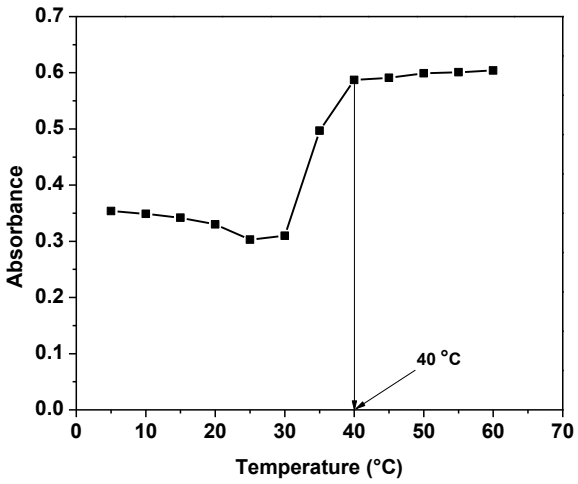


Figure 4. Optimization of reaction temperature for formation of Fe-PPQC chelate complex measured at 418 nm.

The effect of reaction time on the formation of the yellow-colored Fe-PPQC chelate complex was investigated at a fixed concentration of 10.0 µg mL^{-1} of PPQC. The absorbance generated at different reaction times no longer varies noticeably, as seen in **Figure 5**. As a result, it was determined that the optimal duration for the developed process would be 10 minutes.

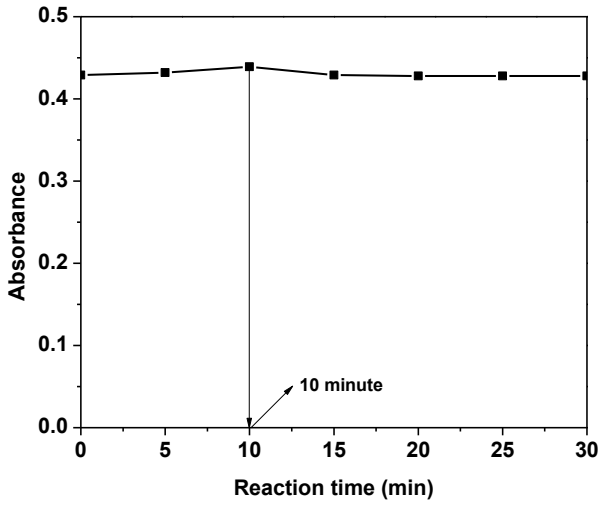


Figure 5. Optimization of reaction time for the formation of the Fe-PPQC chelate complex measured at 418 nm.

3.1.4. Determination of Stoichiometric Ratio for the developed method

The stoichiometry of the reaction between PPQC and $Fe_2(SO_4)_3$was determined by Job's method [30]. Equimolar PPQC and $Fe_2(SO_4)_3$ were utilized during the investigation,. During the investigation, a 10 mL volumetric flask was filled with various volumes of $Fe_2(SO_4)_3$(0.00, 0.40, 0.66, 1.00, 1.34, 1.60, and 2.0), and the remaining volume was filled with PPQC solution to reach 2.0 mL. Then, the resultant solutions were diluted up to 10.0 mL with double-distilled water. At 418 nm, the absorbance was measured against blank reagents that had been made except for the addition of PPQC. The result revealed that one mole of Fe (III) ion can form a co-ordination bond with three moles of PPQC to form a chelate complex. It was found that the maximum value of the mole fraction of the reagent indicates the stoichiometric ratio of PPQC:$Fe_2(SO_4)_3$is 3:1. As shown in **Figure 6**.

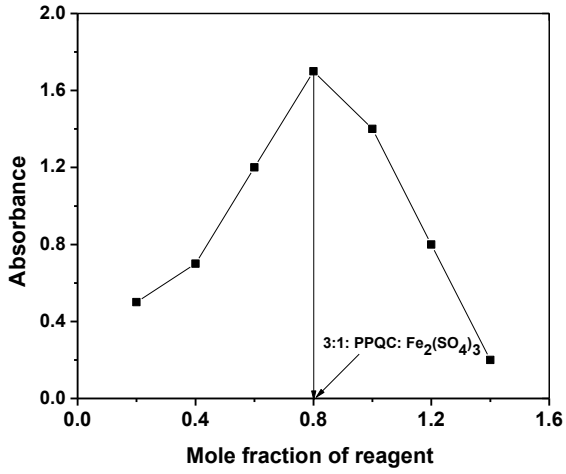


Figure 6. Stoichiometric ratio determination for forming Fe-PPQC chelate complex using equimolar (1.0 mg mL^{-1}) PPQC and $Fe_2(SO_4)_3$ solutions.

3.2. Validation study

The main criteria for evaluating the spectrophotometric system were the linearity test, precision, and accuracy. As per ICH guidelines [31], the proposed method was approved. Calibration curves for the determination of the PPQC were constructed under optimal conditions. Linear calibration curves were found to be in the range of 5-30 µg mL^{-1} for PPQC using double-distilled water as a solvent, 4-14 µg mL^{-1} for paracetamol spiked in blood samples, and 0.2-2 µg mL^{-1} for PPQC spiked in blood samples **(Figures 7-9)**. The calibration curves for PPQC using double-distilled water as the solvent-, PPQC spiked in blood samples-, and paracetamol spiked in blood samples demonstrated good linearity with a determination coefficient of less than 0.990. These calibration curves can be used to assess the accuracy of the suggested method and measure the concentration of PPQC in blood samples for pharmacokinetic studies.

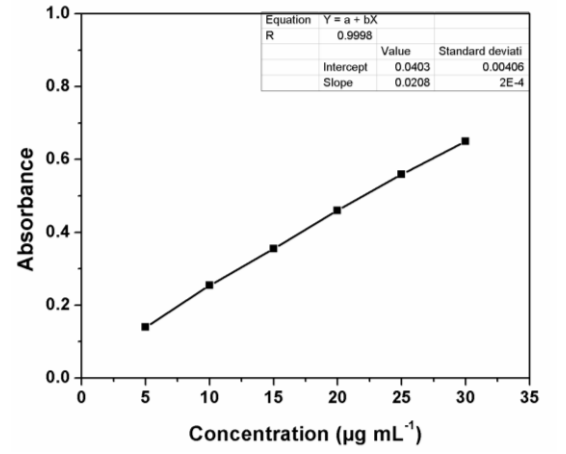


Figure 7. The calibration curve of PPQC in a double distilled water solvent.

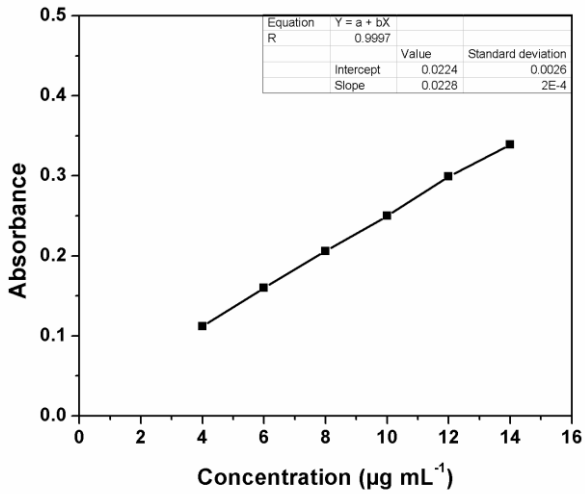


Figure 8. The calibration curve of spiked PPQC in a blood sample.

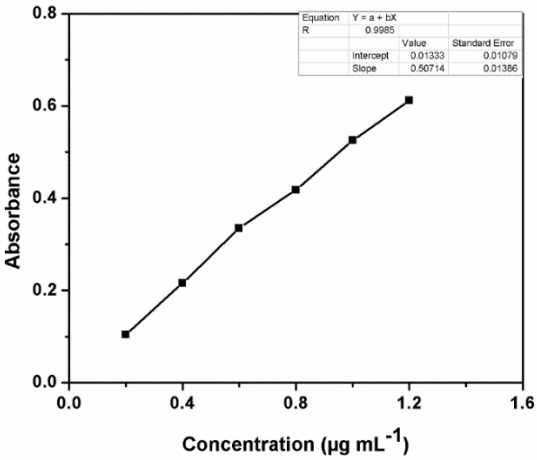


Figure 9. The calibration curve of spiked paracetamol in a blood sample.

The linear regression was generated using the least squares approach. The formula Y = mx + c, in which x stands for concentration, m for slope, c for intercept, and Y for absorbance. The limits of detection (LOD) and quantification (LOQ) were calculated using standard formulas; LOD = 3.3 [standard deviation/slope] and LOQ = 10 [standard deviation/slope]. **Table 2** shows the developed method's correlation coefficient (r), 95% confidence limit, Molar absorptivity, Sandell sensitivity, limit of detection (LOD), and limit of quantification (LOQ).

Table 2. Regression and analytical parameters of the proposed method.

Parameters	Results
Λ_{max}	418 nm
Linear response (µg mL^{-1})	5-46
Sandell's sensitivity (µg cm^{-2})	7.27×10^{-4}
Regression equation	Y= 0.0208x+0.0403
Slope (m)	0.0208
Intercept (c)	0.0403
Correlation coefficient (r)	0.9998
Molar absorptivity (L mol^{-1} cm^{-1})	1.2967×10^{4}
LOD (µg mL^{-1})	0.69
LOQ (µg mL^{-1})	2.09
95% confidence limit	Y = 0.0208x±0.0002+ 0.0403±0.0040

3.2.1. Precision and Accuracy

By examining three duplicate samples of each concentration, the accuracy and precision of the proposed method were assessed at three PPQC concentration levels in blood samples (1.25, 2.50, and 3.75 µg mL^{-1}) [29]. **Table 3** provides an overview of the accuracy and precision of the method developed within and between days. The CV, obtained by repeating the test three times on the same day for intraday precision, was used to evaluate the repeatability of the developed method. The assay of the sample sets on six consecutive days allowed for evaluating of the method's inter-day precision. Similarly intra-day, inter-day accuracy was tested using blood samples with the addition of three known quantities of PPQC. The PPQC in blood samples was extracted using traditional deproteinization [32, 33]. The resulting mixtures were examined using the proposed technique. The percentage recovery for inter-day and intra-day analyses was 98.40–101.60% for blood samples.The linear regression equation and 95% confidence limit for spiked PPQC in blood samples were Y= 0.0228±0.0002X+0.0224±0.002. It was found that the blood sample CV was less than 2.07%. Thus, results show that the devised procedure was more accurate and precise.

3.2.2. Robustness

Variations in wavelength (418±1 nm), working reaction temperature (40±2 °C), and reaction time (10±3 min) were used to assess the robustness of the developed method. PPQC was used at two concentrations (5.0 and 10.0 µg mL^{-1}) to assess the method's robustness. **Table 4** demonstrates the developed method's low CV values (less than 1%), indicating its robustness.

Table 3. Intra- and Inter-day precision and accuracy for determining PPQC in spiked blood samples.

Spiked drug concentration of PPQC in a blood sample (µg mL^{-1})	Intra-day			Inter-day		
	Mean found concentration (µg mL^{-1})	Accuracy* %	Precision (CV)*	Mean found concentration (µg mL^{-1})	Accuracy* %	Precision (CV)*
1.25	1.23±0.07	98.40±0.40	0.41	1.27±0.05	101.60±1.67	1.67
2.50	2.51±0.02	100.40±0.25	0.25	2.49±0.07	99.60±0.80	0.80
3.75	3.74±0.01	99.73±1.80	1.80	3.80±0.09	101.33±0.81	2.07

* Results are the mean of three replicate samples

Table 4. Robustness of the proposed method.

Concentration (µg mL^{-1})		Wavelength (nm)	Absorbance	SD (n=3)	CV
5		417	0.140 ±0.01		
		418	0.139±0.06	0.0006	0.41
		419	0.139±0.10		
10		417	0.255±0.14		
		418	0.254±0.06	0.001	0.39
		419	0.253±0.02		
Temperature (˚C)	10 (µg mL^{-1})	38	0.587±0.04		
		40	0.591±0.02	0.0035	0.59
		42	0.594±0.03		
Reaction time (min)	10 (µg mL^{-1})	7	0.429±0.10		
		10	0.429±0.09	0.0006	0.13
		13	0.428±0.04		

n = values of the mean of three determinations

3.2.3. Ruggedness

The ruggedness of the established method was examined in the same laboratory condition by various analysts over six consecutive days using day-to-day analysis. The ruggedness of the developed method was evaluated using spiked PPQC in blood samples (2.5 µg mL^{-1}) and PPQC at two different concentration levels (5.0 and 10.0 µg mL^{-1}). The outcomes show that the suggested method is reliable, with low CV values (less than 1%; **Table 5**).

Table 5. Ruggedness of the proposed method.

Concentration (µg mL^{-1})	Analyst	Absorbance	SD (n=3)	CV
5.0	I	0.139±0.05	0.0007	0.51
	II	0.138±0.01		
10.0	I	0.254±0.06	0.0014	0.56
	II	0.252±0.08		

n = values of the mean of three determinations, m= six consecutive days

3.2.4. Stability Study

Freeze-thaw, bench top (room temperature for 6–24 hrs), and long-term (–20 ºC for 5 weeks) investigations were conducted to examine the stability of iron chelate formation with spiked PPQC in blood samples. Freshly prepared spiked drugs in blood samples were contrasted with the stability conditions. The bench-top stability test revealed that the spiked blood samples (1.25, 2.50, and 3.75 µg mL^{-1}) were stable for at least 14 hours. The studied drug showed stability in spiked blood samples when stored at -20 ℃ for 5 weeks and long-term stability when compared with the freshly prepared sample. **Table 6** presents a summary of the obtained results. Under storage conditions, the spiked blood samples are relatively stable.

3.2.5. Specificity/ Selectivity

The ability of a method to precisely and selectively identify the analyte of interest in the presence of other components in a sample under the specified conditions of that method is referred to as specificity or selectivity. The method's specificity and selectivity were evaluated by introducing a specific amount of PPQC (10.0 µg mL^{-1}) to each species. The level of interference was regarded as acceptable if the % relative error was 10% or lower. The outcomes are shown in **Table 7**. It was discovered that the % relative error was less than 10% for many species. Consequently, no disruptions to the PPQC study in the presence of the common species were discovered.

Table 7. Determination of (10.0 µg mL^{-1}) PPQC in the presence of common interferents.

Coexisting substance	Ratio of Coexisting substance to APSA	% Relative error
Ca^{2+} (Cl^-)	1:75	1.7
Na^+ (Cl^-)	1:150	0.8
K^+ (Cl^-)	1:75	1.2
Mg^{2+} (Cl^-)	1:0.4	4.8
Zn^{2+} (Cl^-)	1:0.4	0.6
Fe^{2+} (Cl^-)	1:0.4	5.1
L-Alanine	1:75	1.7
Glycine	1:25	0.5
Tyrosine	1:25	3.4
Uric acid	1:0.025	0.1
Paracetamol	1:1	3.0
L-ascorbic acid	1:1	4.1

Table 6. Summary of stability of spiked PPQC in blood samples at varying conditions.

Spiked drug concentration (µg mL^{-1})	Intra-day Accuracy %	Three thaw cycles	% CV	Bench-top stability	% CV	Long-term Stability	% CV
1.25	98.40±0.40	97.38±1.43	0.92	96.54±2.73	5.53	100.1±1.47	3.27
2.50	100.40±0.25	98.13±2.25	1.12	98.16±0.86	0.88	99.70±0.64	1.54
3.75	99.73±1.80	97.58±0.90	3.87	95.29±3.49	1.34	97.16±1.73	5.10

n= values of the mean of three determinations

3.3. Pharmacokinetics study

The pharmacokineticsparameters for the synthesized compound PPQC were determined using the trapezoidal rule and the non-compartment animal model [34-36].Pharmacokinetics data for PPQC were determined by adeveloped spectrophotometric method and compared with standard paracetamol drugs using blood samples. The reported method was used to evaluate the pharmacokinetics study for paracetamol drug [1]. The amounts of PPQC and paracetamol from blood samples of Wistar albino were calculated using the regression equation and calibration curves depicted in **Figures 7-9**. The regression equations for paracetamol and PPQC in blood serum were Y = 0.0228X + 0.0224 (r = 0.9997) and Y = 0.5071X + 0.0133 (r = 0.9985), respectively.

Table 8 summarizes of the pharmacokinetic parameters assessed for PPQC and paracetamol drugs in the blood sample.

Table 8: Determine the pharmacokinetics parameters (Mean ± SD) for synthesized PPQC and paracetamol in spiked blood samples.

Pharmacokinetic parameter	Paracetamol	PPQC
Tmax (hr)	1.0 ± 0.00*	3.0 ± 0.00*
Cmax (µg mL^{-1})	5.18 ± 0.57*	2.54 ± 0.28*
MRT (hr)	3.95 ± 0.16*	7.06 ± 2.08*
Kel (hr^{-1})	0.25 ± 0.01*	0.15 ± 0.04*
$t_{1/2}$ (hr)	2.74 ± 0.11*	4.89 ± 1.44*
$AUC_{(0-\infty)}$ (µg hr mL^{-1})	20.51 ± 1.79*	13.33 ± 1.98*
$AUMC_{(0-\infty)}$(µg hr^2 mL^{-1})	80.97 ± 6.72*	96.18 ± 39.28*
CL/F (L hr^{-1} kg^{-1})	7.43 ± 0.89*	13.94 ± 2.63*
Vss/F (L kg^{-1})	38.30 ± 2.13*	27.46 ± 8.01*
F	-	0.65

Tmax = Time corresponding to maximum drug concentration; Cmax = Maximum drug concentration; MRT = Mean residence time; Kel = Elimination rate constant; $t_{1/2}$ = Elimination half-life; $AUC_{(0-\infty)}$ &$AUMC_{(0-\infty)}$ = Area under the drug concentration curve and area under the first moment of drug concentration curve from time 0 to ∞; CL = Oral clearance; Vss = Apparent volume distribution at equilibrium after oral administration; F = Relative bioavailability.

Serum concentrations at different sampling times exhibit a clear decline, as indicated by the concentration-time profiles of PPQC and paracetamol **(Figure 10)**. **Figure 10** describes the mean concentration profile of paracetamol and PPQC. $AUC_{0-\infty}$values for PPQC were 13.33 ± 1.98 µg hr mL^{-1}, which is significantly lower than standard paracetamol (20.51 ± 1.79 µg hr mL^{-1}) **(Figure 11)**.T_{max} also shows how quickly a drug is absorbed. Because PPQC had higher T_{max} values (3.0 hr), it was discovered that its rate of absorption was lower than that of paracetamol (1 hour) **(Figure 10)**. Furthermore, the C_{max} value of PPQC (C_{max} = 2.54 ± 0.28 µg mL^{-1}) was found to be lower than that of paracetamol (C_{max} = 5.18 ± 0.57 g mL^{-1}), suggesting that PPQC has a weaker therapeutic and toxic response.

The MRT for PPQC (7.06 ± 2.08 hrs) was significantly higher than the MRT for paracetamol (3.95 ± 0.16 hrs), suggesting that PPQC stayed in the body for extended periods **(Figure 11-13)**. PPQC had a significantly lower elimination rate constant (Kel) (0.15 ± 0.04 hr^{-1}) than paracetamol (0.25 ± 0.01 hr^{-1}) **(Figure 10)**.

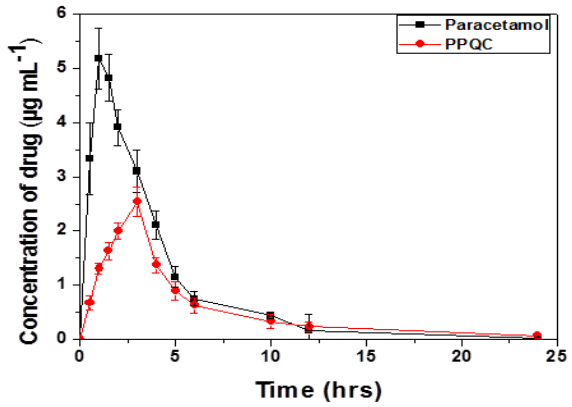


Figure 10. Mean drug concentration vs. time curves in rats after oral administration of 500 and 600 mg kg^{-1} of paracetamol and PPQC.

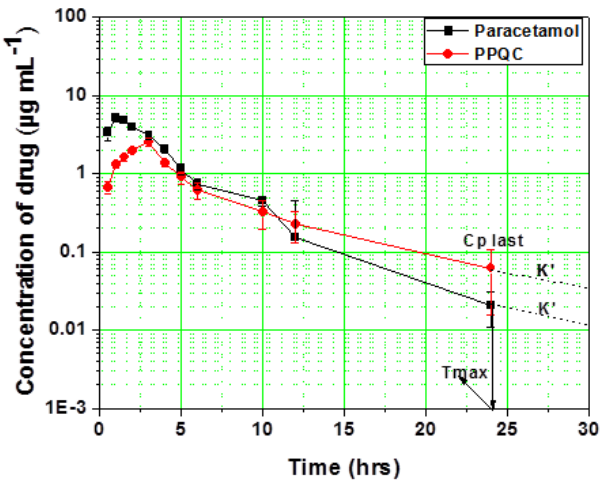


Figure 11. Semi-log curves of mean drug concentration vs. time after oral administration of 500 and 600 mg kg^{-1} of paracetamol and PPQC in rats.

The elimination rate constant (Kel) results showed that PPQC was removed more slowly than paracetamol from the rat's body. PPQC was found to have a significantly longer elimination half-life ($t_{1/2}$) (4.89 ± 1.44 hrs) than paracetamol (2.74 ± 0.11 hrs) **(Figure 10)**. The apparent volume distribution (Vss/F) of PPQC was found to be significantly lower (27.46 ± 8.01 L kg^{-1}) compared to paracetamol (38.30 ± 2.13 L kg^{-1}). The apparent volume distribution analysis showed that the synthesized compound PPQC was less distributed in the rat body than paracetamol **(Figure 11, 13)**. Relative bioavailability was calculated using the $AUC_{0-\infty}$ of PPQC; it was found to be 0.65. Based on previous reports, the bioavailability of PPQC is lower than that of paracetamol, which ranges from 60 to 90% [37].

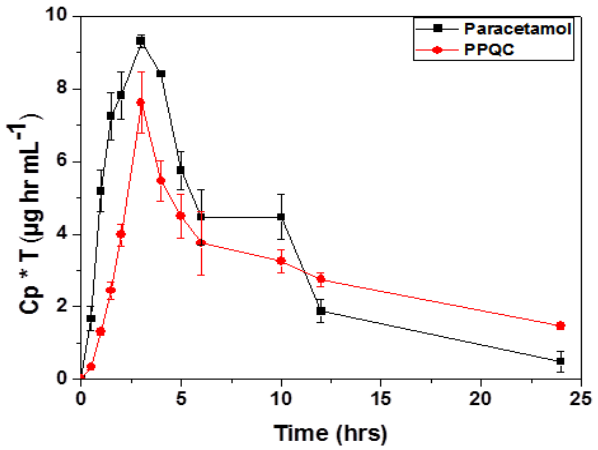


Figure 12. The area under the first moment curves after oral administration of 500 and 600 mg kg^{-1} of paracetamol and PPQC in rats.

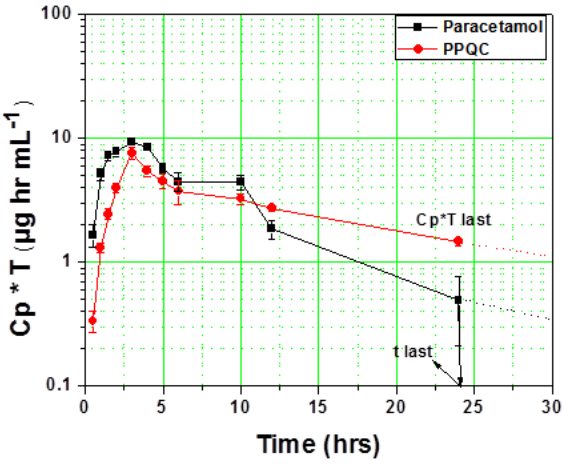


Figure 13. The area under the first moment semi-log curves after oral administration of 500 and 600 mg kg^{-1} of paracetamol and PPQC in rats.

4. Conclusion

Since no other analytical method for evaluating PPQC in blood samples has been published in the literature, the spectrophotometric technique developed is unique. A spectrophotometric approach was developed for PPQC using a Fe-PPQC chelate complex formation method. The developed method is optimized and validated for the PPQC compound. Common interfering species did not interfere with determining the PPQC compound. It demonstrates the specificity of the suggested approach for PPQC compound determination. The spectrophotometric method developed is precise, accurate, and reliable. However, when determining the concentration of PPQC below five µg mL^{-1}, the suggested method is not applicable. This represents a constraint of the suggested approach. Overall the main advantages of the recommended method are a shorter analysis time, less reagent volume, and less solvent usage. The expensive facilities and equipment are not required for the suggested method.The proposed technique can be utilized for therapeutic medication monitoring in clinical laboratories and routine analysis to determine PPQC.

Conflicts of Interest

The authors disclose no conflicts of interest.

Athors Orcid numbers:

Pravin S. Patil: 0009-0007-2506-9281
Khanderao Pagar: 0009-0003-9859-1389
Pramod Patil: 0009-0006-2169-4728

Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

References

1. Issa MM, Nejem RM, El-Abadla NS, Al-Kholy M, Saleh AA. Novel atomic absorption spectrometric and rapid spectrophotometric methods for the quantitation of paracetamol in saliva: application to pharmacokinetic studies. Indian journal of pharmaceutical sciences. 2008 May; 70(3):344-350
2. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-visible spectrophotometric method

development and validation of assay of paracetamol tablet formulation. Int. J. Pharm. Sci. Res.(2012) 3 (12): 4945-4953.

3. Shah B, Patil P, Shah H. Chemical modification of paracetamol and their antimicrobial and pharmacological evaluation. Int. J. Pharm. Res. Bio-Sci. (2014) 3(2): 12-31.
4. Shah B, Patil P, Shah H. Synthesis, Pharmacological Activity and Spectrophotometric Determination of [Di (4-amino N-acetyl) phenoxy] methyl ketone in Biological Fluids. Int. J. Res. Pharm. Biomed. Sci.(2013) 4(4): 1038-1050.
5. Al-Swayeh OA, Futter LE, Clifford RH,Moore PK. Nitroparacetamol exhibits anti-inflammatory and anti- nociceptive activity. Br. J. Pharmacol.(2000) 130 (7): 1453-1456.
6. Profire L, Sunel V, Lupascu D, Baican MC, Bibire N, Vasile C. New Theophylline Derivatives with Potential Pharmacological Activity. Farmacia. (2010) 58(2): 170-176.
7. IwuozorKO. A Review on the Properties and Uses of Paracetamol. Int. J. Pharm. Chem.(2019) 5 (3): 31-35.
8. Farid NF, Abdelaleem EA. HPTLC method for the determination of paracetamol, pseudoephedrine and loratidine in tablets and human plasma. J. Chromatogr. Sci.(2016) 54 (4):647–652.
9. Ambekar A, KuchekarB. Application of a validated stability-indicating HPTLC method for simultaneous estimation of paracetamol and aceclofenac and their impurities. J. Chromatogr. Sep. Technol.(2016) 7 (3):324–334.
10. Belal T, Awad T, Clark R. Determination of paracetamol and tramadol hydrochloride in pharmaceutical mixture using HPLC and GC-MS. J. Chromatogr. Sci.(2009) 47 (10):849–854.
11. Sharma H, Vishakha K, Kumar KV, Bhatta HP. Validated RP-HPLC method for simultaneous estimation of paracetamol, pamabrom and dicyclomine, hydrochloride in bulk and pharmaceutical dosage form. Int. J. Pharm. Sci. Res.(2016) 7 (1):316–324.
12. Nazir A, Naseer Y, Shahid R, Raza S. Development & validation of analytical method used for simultaneous determination of paracetamol, caffeine and codeine phosphate by HPLC, in pharmaceutical formulation. Sci. Int.(2016) 28 (3):2497-2499.
13. Li H, Zhang C, Wang J, Jiang Y, Fawcett JP,Gu J. Simultaneous quantitation of paracetamol, caffeine, pseudoephedrine, chlorpheniramine and cloperastine in human plasma by liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal.(2010) 51 (3):716–722.
14. Emre D, Özaltın N. Simultaneous determination of paracetamol, caffeine and propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography. J. Chromatogr. B Anal. Technol. Biomed. Life Sci.(2007) 847 (2):126–132.
15. Demirhan N, Tuncel Elmali F. Spectrophotometric Determination of Iron (II) with 5-Nitro-6-amino-1, 10-phenanthroline. Turk. J. Chem.(2003) 27(3): 315-321.
16. Topuz B, Adanur SM, Yalcuk A. A new method for simultaneous determination of trace amounts of Cu (II) and Ni (II) ions by preconcentration and spectrophotometric analysis. Turk. J. Chem.(2017) 41 (5): 619-629.
17. Quifen HU, Yang G, Dong X, Yin J. Study on the solid phase extraction andspectrophotometric determination of Cobalt with 2-(2-Quinolylazo)-5-Diethylaminoaniline. Turk. J. Chem. (2004) 28 (5): 611-619.
18. Soomro R, Ahmed MJ, Memon N. Simple and rapid spectrophotometric determination of trace level chromium using bis (salicylaldehyde) orthophenylene diamine in nonionicmicellar media. Turk. J. Chem. (2011) 35 (1): 155-170.
19. Belal S, Abdel-Razak O, El-Walily AF, Bakry R. Spectrophotometric determination ofCaptopril and Penicillamine through the use of ligand exchange complexation reactions. Int. J. Biomed. Sci. (2011) 7 (4): 289-294.
20. Bhardwaj N, Kaushik J.Spectrophotometric study of complexation of Labetalol withsome transition metal ions. World J. Pharm. Pharm. Sci.(2017) 6(3): 1108-1111.
21. Rajendraprasad N, Basavaiah K. Modified spectrophotometric methods fordetermination of Iron (III) in leaves and pharmaceuticals using salicylic acid. Indian J. Adv. Chem. Sci.(2016), 4(3):302-307.

22. Al-Nuzal SMD, Al-Oba YM, Al-Asadi AH. Analytical study of Al(III)-Tetracyclinecomplex for drug formulations. J. Appl. Chem.(2014) 3(4): 1592-1601.
23. El-Bagary RI, Elkady EF, Farid NA, Youssef NF. A Validated Spectrophotometric Method and Thermodynamic Studies for the Determination of Cilostazol and Rivaroxaban in Pharmaceutical Preparations Using Fe-Phenanthroline System. Anal. Chem. Lett.(2017) 7(5): 676-688.
24. Azmi SNH, Iqbal B, Jaboob MAM, Al Shahari WAS, Rahman N. Spectrophotometric Determination of Piroxicam via Chelation with Fe (III) in commercial Dosage Forms. J. Chin. Chem. Soc. (2009) 56 (6): 1083-1091.
25. Lutfullah, Sharma S, Rahman N, Azmi SNH, Hidaifi HJS, Alquasmi MMA.Spectrophotometric determination of Fe(III) via complexation with piroxicam in syntheticmixture and soil samples. J. Sci. Ind. Res.(2010) 69(2): 135-141.
26. Al-Hammoodi IAS, Al-Enizz MS, Saeed AM. A. Spectorphotometric Methods forDetermination of Terbutaline Sulfate in Pure and Pharamaceutical Formulation. J.Med. Chem. Sci.(2023) 6 (5): 1032-1043.
27. Salama F, El-Abasawy N, Razeq SA, Ismail MM, Fouad MM. Validation of the spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates. Journal of pharmaceutical and biomedical analysis. 2003 Oct 15;33(3):411-421.
28. Gaber M, Khedr AM., El-Kady A. S. Spectrophtometric determination of norfloxacin inpureand dosage forms by complexation with Fe(III) and Cu(II) ions. Int. Res. J. Pharm. Pharmacol.(2012)2(5): 97-102.
29. Guide for Industry,Bioanalytical Method Validation, U.S Department of Health andHuman Services Food and Drug Administration (FDA), (2001).
30. Bosque-Sendra JM, Almansa-Lopez E, Garcia-Campana AM, Caudros-Rodriguez L.Data analysis in the determination of stoichiometries and stability constants ofcomplexes. Anal. Sci. (2003) 19 (10): 1431-1439.
31. ICH Q2(R2), Validation of Analytical Procedures: Text and Methodology,adopted in November 2023.
32. West JC. Rapid HPLC Analysis of Paracetamol (Acetaminophen) in Blood andPostmortem Viscera. J. Anal. Toxicol.(1981)5 (3), 118-121.
33. Patil P, Machhi J. Development and validation of spectrophotometric method forestimation of sodium [2-[2,6-dichlorophenyl[(4-amino N-acetyl)phenyl acetate]-amino]phenyl acetate] in rat serum and its application in pharmacokinetics studies. Int. J. Chem. Sci.(2018) 2(6): 19-29.
34. Gibaldi MI, Perrier DO. Pharmacokinetics (2nd edn). Marcelly Dekker: New York. 1982. 121-123.
35. PHAR 7633 Basic Pharmacokinetics, Chapter 20, Non Compartmental Analysis. (2012) 1-10.
36. Fajdetic A, Vinter A; Paljetak HC, Padovan J, Jakopovic IP, Kapic S.Synthesis, activity and pharmacokinetics of novel antibacterial 15-membered ring macrolones. Eur. J Med. Chem.(2011) 46(8):3388-3397.
37. Oscier CD, Milner QJ. Peri-operative use of paracetamol. *Anaesthesia*.(2009) 64(1):65-72.