

Indirect Spectrophotometric Determination of Piroxicam by Using Acid Fuchsine Dye

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Abstract :

A rapid, sensitive, and precise indirect spectrophotometric method has been developed to determine Piroxicam. This method involves the oxidation of the Piroxicam using excess N-bromosuccinimide (NBS), and then the unreacted NBS reacts with acid fuchsine dye to produce a pink-colored product. The absorbance of this product is measured spectrophotometrically at 544 nm. Under optimized experimental conditions, the method followed Beer's law within a Piroxicam concentration range of 2.5–20 µg/mL. The detection limit was 0.421 µg/mL, and the quantification limit was 1.277 µg/mL. The method demonstrated high sensitivity, with a sandal index of 0.0232 µg/cm² and a molar absorptivity of 1.4248×10^4 L/mol.cm. The relative standard deviation (RSD) was less than 1.5%. It has been successfully applied to determine Tablet Piroxicam content and can be extended to other formulations containing the drug. This method is highly suitable for quality control and routine analysis.

Key Words: Piroxicam (PIX), Acid Fuchsine Dye, N-bromosuccinimide (NBS).

التقدير الطيفي الغير المباشر للبىروكسيكام باستخدام صبغة الفوكسين الحامضية

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مستخلص:

طُوّرت طريقة طيفية غير مباشرة سريعة وحساسة ودقيقة لتقدير البىروكسيكام. تتضمن هذه الطريقة أكسدة البىروكسيكام باستخدام زيادة من ن-بروموكسينيميد، ثم مفاعلة الفائض منه مع صبغة الفوكسين الحامضية وقياس المتبقى من الصبغة الوردية عند الطول الموجي 544 نانومتر. اتبعت الطريقة قانون بير ضمن مدى تراكيز يتراوح من 2.5 - 20 ميكروغرام/مل. كان حد الكشف 0.421 ميكروغرام/مل، والحد الكمي 1.277 ميكروغرام/مل. أظهرت الطريقة حساسية عالية، حيث بلغ دلالة ساندل 0.0232 ميكروغرام/سم²، وبلغت الامتصاصية مولارية 1.4248×10^4 لتر/مول.سم. كان الانحراف المعياري النسبي (RSD %) أقل من 1.5%. وقد طبقت بنجاح لتقدير محتوى أقراص بىروكسيكام، ويمكن توسيع نطاقها لتشمل تركيبات أخرى تحتوي على الدواء. تُعد هذه الطريقة مناسبة للغاية لمراقبة الجودة والتحليل الروتيني.

الكلمات المفتاحية: بىروكسيكام، صبغة الفوكسين الحامضية، ن-بروموكسينيميد.

Introduction

Piroxicam, an oxicam-class NSAID (1,2), is structurally depicted in Figure 1. It is prescribed for musculoskeletal and joint disorders, including osteoarthritis, rheumatoid arthritis, juvenile idiopathic arthritis, acute gout, and postoperative pain (3–5). Studies indicate that combining 40 mg oral piroxicam with levonorgestrel enhances its efficacy as emergency contraception (6). Emerging research also explores its potential in treating tumors, colorectal cancer, and invasive bladder cancer (7). The drug exerts its anti-inflammatory and analgesic impacts by inhibiting cyclooxygenase (COX-1 and COX-2), thereby reducing prostaglandin synthesis (8,9). However, due to safety concerns, its systemic use in Europe is restricted to chronic inflammatory and painful conditions (10). Contraindications include active peptic ulcers, gastrointestinal bleeding, inflammatory bowel disease, and severe heart failure (11). The standard oral dose for rheumatic diseases is 20 mg/day, often split into two administrations. Rectal, intramuscular, and top-

ical (0.5% gel) formulations are also available for short-term or localized treatment (12). The growing demand for NSAIDs underscores the importance of robust pharmaceutical analysis to ensure drug quality, safety, and efficacy (13–15). Analytical techniques such as HPLC (16–21), capillary electrophoresis (22–24), TLC (25–27), and electrochemical methods (28–34) are commonly used for piroxicam quantification. Spectrophotometric assays (35–40) offer a simpler alternative for pharmaceutical analysis. This study introduces a rapid, sensitive spectrophotometric method to quantify piroxicam in pure and pharmaceutical formulations, leveraging stable chromogenic reactions for reliable results.

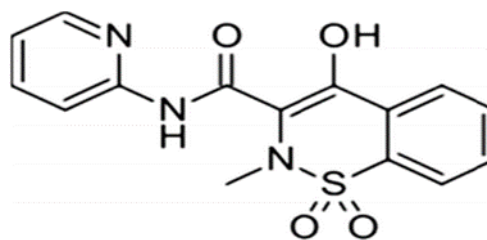


Figure 1:

Chemical structures of piroxicam

The aim of the study:

Given piroxicam's significant therapeutic role in treating pain and in-

flammation, it is essential to develop reliable analytical methods for its quantification. The proposed approach offers a simple, highly sensitive solution for determining piroxicam concentrations in pure form and pharmaceutical formulations. Its accuracy and ease of use make it a practical tool for quality control in drug manufacturing and research.

Apparatus

Absorbance measurements were conducted using a Shimadzu UV-Visible 1800 digital double-beam recording spectrophotometer, employing quartz cells. A KERN-ACS 120-4 sensitive balance (Sartorius BL210S Company, L.C. = 0.0001g) was utilized for weighing purposes.

Reagents

Analytical reagent grade was used for all of the chemicals. The raw materials PIX were obtained from the India Company, which were 99.9%. We obtained sodium hydroxide from Sober Life in Suleimana, Iraq, and the analytical-reagent quality Acid Fuchsin and HCl (36% w/w) were obtained from Fluka. The buffer solution was prepared from NaOH, KCl at 25 °C, con-

taining 25 mL 0.2 M KCL (14.91g/l) and 66 mL 0.2M NaOH, diluted to 100 mL in a volumetric flask [41].

Preparation of Standard and Reagent Solutions

1. Piroxicam Standard Solution (1000 µg/mL)

Method: Dissolved 0.1000 g of piroxicam in 10 mL of pH 13 buffer solution.*

2. Fuchsine Acid Solution (1.7×10^{-4} M)

Method: Prepared by dissolving 0.01 g of fuchsine acid in distilled water.*

3. N-Bromosuccinimide Solution (1×10^{-3} M)

Method: Dissolved 0.0177 g of N-bromosuccinimide in distilled water.*

4. Hydrochloric Acid Solution (~0.1 M)

Method: Diluted 0.84 mL of concentrated HCl (11.98 M) with distilled water.*

***Dilution:** Final volume made up to 100 mL in a volumetric flask.

Piroxicam 20mg Tablet PIROX-EN

To prepare a 100 µg/mL piroxicam solution, six Tablets (total weight: 3.0299 g) were crushed and homogenized, and a 0.1000 g aliquot of the powder (equivalent to 2.5249 g pure piroxicam) was dissolved in 10 mL of buffer solution, followed by filtration and washing. The filtrate was transferred to a 100 mL volumetric flask and diluted to the mark with distilled water. A 10 mL aliquot of this solution was further diluted in another 100 mL volumetric flask to achieve the final 100 µg/mL concentration, ensuring accurate and precise preparation for subsequent analysis. This method ensures proper extraction and dilution while maintaining the sample's integrity.

Principle of Method

The method entails the oxidation of piroxicam in an acidic medium utilizing an excess oxidizing agent. The unreacted oxidizing agent is quantified by reacting with acid fuchsine dye. The intensity of the resulting pink color from the remaining dye is then measured spectrophotometrically at 544 nm. This approach allows for the indirect deter-

mination of piroxicam concentration based on the residual oxidizing agent.

Results and Discussion

Selection of the best amount of dye

A systematic study was conducted using a 100 µg/mL dye solution. Different volumes of the dye (ranging from 0.5 to 4 mL) were diluted to 20 mL in a volumetric flask, and the absorbance was measured at 544 nm. The results (Table 1 and Figure 2) revealed that 3.5 mL of dye gave this wavelength a suitable absorbance value. Consequently, this volume was selected for all further experiments to ensure accuracy and reproducibility.

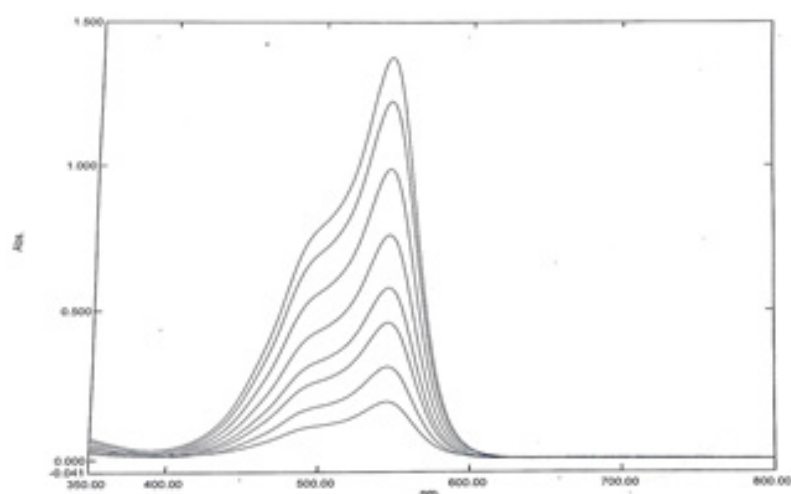


Figure (2) Absorbance spectrum of Acid Fuchsine dye

Table 1: Result of dye absorption

| V(mL) of Acid Fuchsine dye | Absorbance |
|----------------------------|--------------|
| 0.5 | 0.191 |
| 1 | 0.309 |
| 1.5 | 0.457 |
| 2 | 0.575 |
| 2.5 | 0.757 |
| 3 | 0.986 |
| 3.5 | 1.222 |
| 4 | 1.376 |

Selection of the best oxidizing agent

3.5 mL, 100 μ g/mL of acid fuchsine dye was taken, then 3.5 mL, 5×10^{-4} M of different types of oxidizing agents were added, and the solutions were left for 15 minutes to ensure the completion of the oxidation. After that, they

were diluted to the mark with distilled water in a 20 mL volumetric flask. The solutions were measured at 544 nm. The results are shown in Table 2. N-bromosuccinimide was adopted in all subsequent experiments.

Table 2: Selection of the best oxidizing agent

| Type of oxidizing agent | Chemical structure | Molecular weight | Absorbance |
|----------------------------|---|------------------|--------------|
| Without an oxidizing agent | | | 1.222 |
| N-Bromosuccinimide | C₄H₄BrNO₂ | 177.98 | 0.111 |
| Sodium periodate | NaIO ₄ | 213.89 | 0.478 |
| Sodium Iodate | NaIO ₃ | 197.892 | 0.493 |
| N-Chlorosuccinimide | C ₄ H ₄ ClNO ₂ | 133.35 | 0.182 |

Impact of the oxidizing agent amount

The optimal amount of oxidizing agent was determined by evaluating different concentrations and volumes, with the most impactful volume identified as the one yielding the lowest absorbance, corresponding to the greatest reduction in dye color intensity. As

shown in Table 3, 2 mL of 1×10^{-3} M was the most efficient, as it produced the minimal absorbance, and was therefore selected for all subsequent experimental procedures. This optimization ensures maximum oxidative degradation efficiency while minimizing reagent consumption.

Table 3: Impact of oxidizing agent amount

| V(mL) of Oxidizing agent | Absorbance | |
|--------------------------|----------------------|----------------------|
| | M $10^{-4} \times 5$ | M $10^{-3} \times 1$ |
| 0 | 1.222 | 1.222 |
| 0.1 | 1.000 | 0.370 |
| 0.3 | 0.843 | 0.340 |
| 0.5 | 0.722 | 0.325 |
| 1 | 0.588 | 0.288 |
| 1.5 | 0.493 | 0.213 |
| 2 | 0.474 | 0.068 |
| 2.5 | 0.374 | 0.045 |
| 3 | 0.217 | 0.043 |
| 3.5 | 0.069 | 0.042 |
| 4 | 0.035 | 0.042 |

Impact of acid quantity

The Impact of different concentrations of hydrochloric acid on the reaction was studied, where different volumes of acid at concentration 0.1N were added to 3.5 mL of acid fuchsine dye with a concentration of 1.7×10^{-4} M. Then, 2 mL of oxidizing agent with

a concentration of 1×10^{-3} M was added, and the solutions were left for 20 min. Then the absorbance of each solution was measured at 544 nm. The results are shown in Table 4, and a volume of 1 mL of acid was adopted as the optimum volume.

Table 4: Impact of the Acid Quantity

| Hydrochloric Acid V(mL) of | Absorbance |
|----------------------------|--------------|
| 0.5 | 0.127 |
| 1 | 0.150 |
| 1.5 | 0.159 |
| 2 | 0.160 |
| 2.5 | 0.162 |
| 3 | 0.163 |

Study of the time required to complete drug oxidation.

To establish the time required for complete oxidation of the drug, 2 mL of a 1×10^{-3} M oxidizing agent was added to 2 mL of the drug solution (100 $\mu\text{g}/\text{mL}$) in an acidic medium containing 1 mL of 0.1 M hydrochloric acid. The reaction mixtures were allowed to stand for varying time intervals before adding 3.5 mL of acid fuchsine dye (1.7×10^{-4} M). After each interval, the

solutions were left for 20 minutes, diluted to the final volume with distilled water, and their absorbance was measured at 544 nm. The results (presented in Table 5) indicated that 5 minutes was the optimal oxidation time, ensuring complete drug reaction before dye addition. This duration was subsequently used in all further experiments.

Table 5: Impact of the time required to complete the oxidation of the drug

| Time(min) | Absorbance |
|-----------|------------|
| 0 | 0.401 |
| 5 | 0.423 |
| 10 | 0.422 |
| 15 | 0.427 |
| 20 | 0.421 |

Study of the stability of the resulting product

The stability of the product formed was studied for three different concentrations of piroxicam (5, 10, 15µg/mL),

where the absorbance was measured at 544 nm. Table 6 below shows that the colored product is stable for over an hour.

Table 6: Results of the stability of the product

| µg/mL of drug | 0 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 5 | 0.266 | 0.251 | 0.247 | 0.236 | 0.238 | 0.240 | 0.243 | 0.239 | 0.241 | 0.238 | 0.240 | 0.240 |
| 10 | 0.491 | 0.463 | 0.444 | 0.417 | 0.422 | 0.425 | 0.426 | 0.422 | 0.423 | 0.421 | 0.422 | 0.423 |
| 15 | 0.682 | 0.651 | 0.641 | 0.634 | 0.638 | 0.637 | 0.644 | 0.641 | 0.642 | 0.636 | 0.640 | 0.641 |

Calibration Curve

A series of 20 mL volumetric flasks was prepared with incremental additions (0.5-5.5 mL) of the 100 µg/mL drug solution. To each flask, 2 mL of 1×10^{-3} M oxidizing agent and 1 mL of 0.1 M HCl were added. Following a 5-minute oxidation period, 3.5 mL of acid fuchsine dye (1.7×10^{-4} M) was

introduced. After thorough mixing, the reaction mixtures stood at room temperature for 20 minutes before final dilution to the 20 mL mark with distilled water. The resulting solutions were analyzed spectrophotometrically at 544 nm, with the calibration data presented in Figures 3 and 4. The method demonstrated linearity over the concentration

range of 2.5-20 $\mu\text{g/mL}$, as evidenced by a correlation coefficient of 0.9967. The molar absorptivity was determined to be $1.4248 \times 10^3 \text{ L/mol}\cdot\text{cm}$, with a

Sandell's sensitivity of $0.0235 \mu\text{g/cm}^2$, confirming the method's suitability for quantitative analysis.

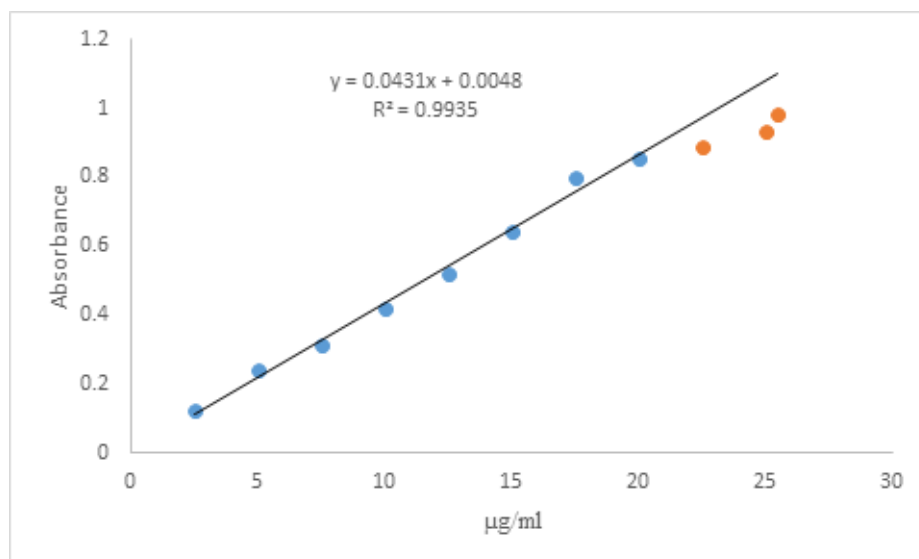


Figure 3. Calibration curve of Piroxicam

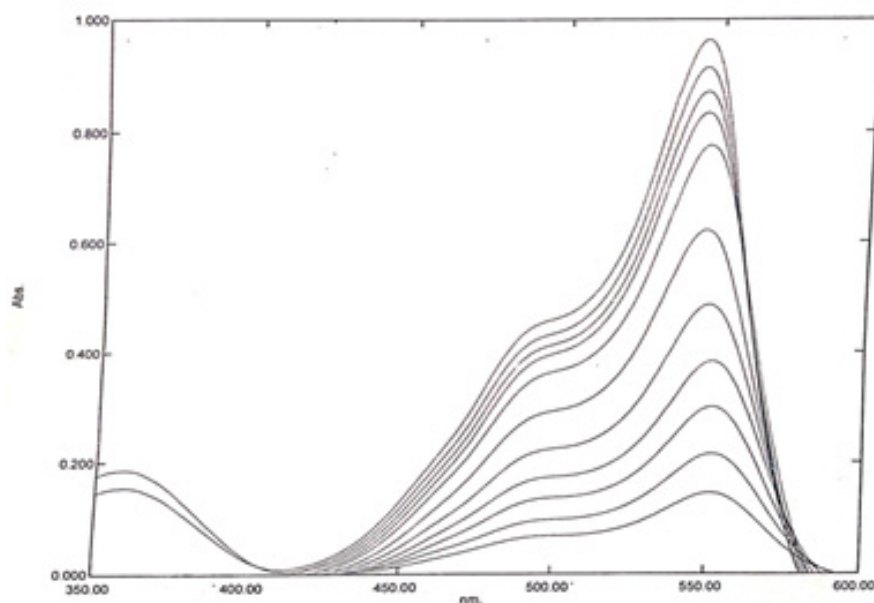
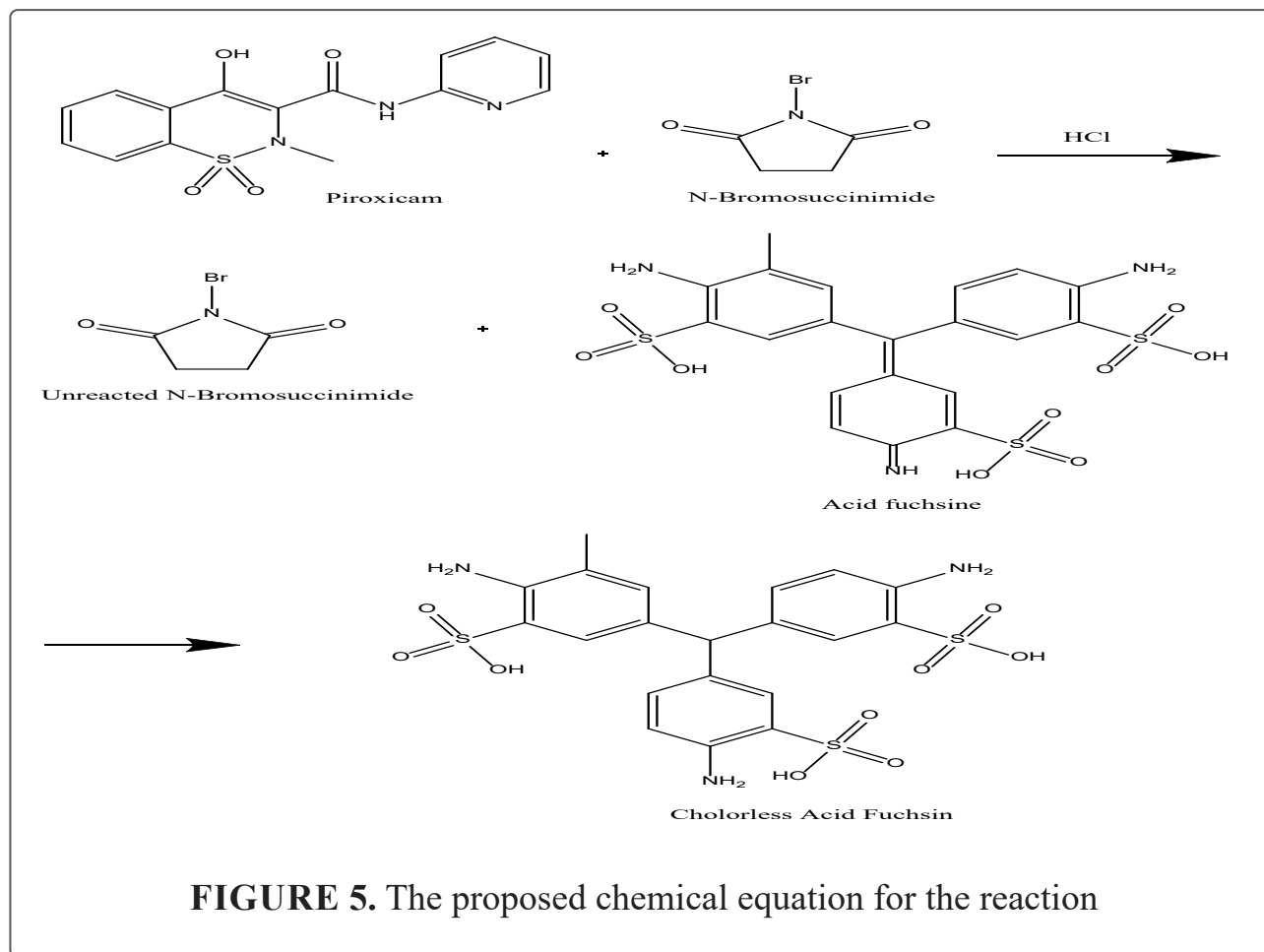


FIGURE 4. The absorption spectrum of concentrations 2.5– 20 $\mu\text{g/mL}$ of PIX

The proposed chemical equation for the reaction [42,43]



FINAL ABSORPTION SPECTRUM

The experimentally determined op-

timal parameters for the spectrophotometric determination of piroxicam are

systematically presented in the Table 7.

Table 7: Summary of optimal conditions for the determination of piroxicam

| Experimental conditions | |
|--|--------|
| λ_{\max} | nm 544 |
| Amount of $5 \times 10^{-3} \text{M}$ oxidizing agent | ml 2 |
| Amount of $1.7 \times 10^{-4} \text{ mol.L}^{-1}$ of dye | ml 3.5 |
| Amount of 0.1 M hydrochloric acid | ml 1 |
| Oxidizing time | min 20 |
| Solvent | Water |
| Temperature | C° 25 |

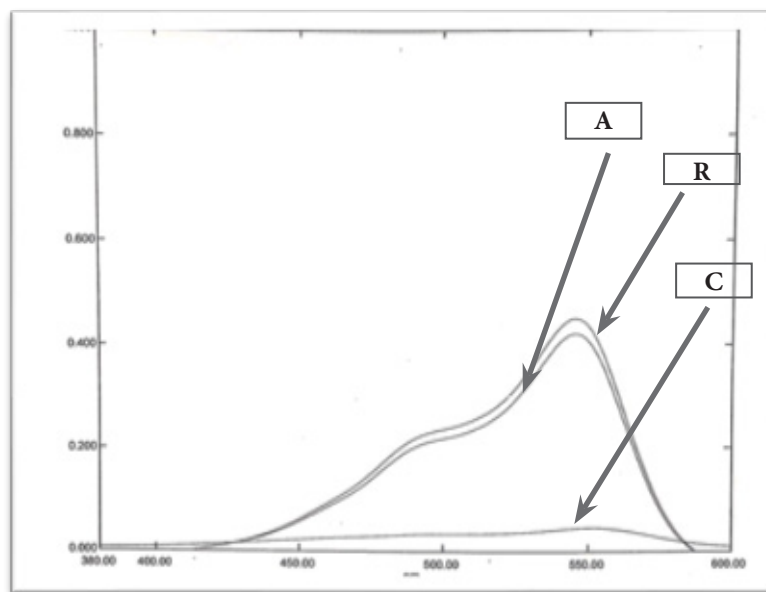


FIGURE 6. Ultimate absorption spectrum of the colored product

A: Displays the absorption spectrum of a piroxicam solution measured using distilled water as the reference.

B: Shows the absorption spectrum of the same piroxicam solution, but measured against the blank solution.

C: Indicates the absorption spectrum of the blank solution when referenced against distilled water.

AACCURACY AND PRECI- SION (98.2-101.8%)

The optimized analytical conditions were subsequently employed to validate the method's performance characteristics. Through rigorous testing, the procedure demonstrated excellent analytical reliability, as evidenced by:

1. High percentage recovery rates

2. Minimal relative standard deviation (RSD < 1.5%)

These validation parameters, detailed in Table 8, confirm the method's compliance with International Council for Harmonisation (ICH) guidelines.

Table 8. Accuracy and precision of the method.

| Amount of Piroxicam Taken µg/mL | Amount of Piroxicam Found µg/mL | % ,RE | ,Recovery % | Average Recovery | % ,RSD |
|---------------------------------|---------------------------------|-------|-------------|------------------|--------|
| 7.5 | 7.2 | 3.7 | 96.2 | 98.3 | 1.28 |
| 15 | 15.0 | -0.23 | 100.2 | | 0.516 |
| 20 | 19.7 | 1.48 | 98.5 | | 0.670 |

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined using established mathematical formulas, with

$LOD = 3.3 \times \delta/S$ and $LOQ = 10 \times \delta/S$, where S represents the slope of the calibration curve (0.0431) and δ is the standard deviation of the intercept

(0.0048) obtained from four replicate measurements of the regression equation ($y = 0.0431x + 0.0048$). These calculations ensure the lowest detectable and quantifiable analyte concentrations, providing critical validation parameters for the analytical method. The derived values in Table 9 confirm the method’s sensitivity and reliability in trace-level detection.

Table 9: Results of the study LOD and LOQ.

| *Slope | * δ | LOD µg/mL | LOQ µg/mL |
|--------|------------|-----------|-----------|
| 0.0431 | 0.0048 | 0.421 | 1.277 |

*The average slope and intercept were determined by repeating the calibration curve four times.

APPLICATIONS

Direct Measurement Method

Different volumes (1.5, 3, 4 mL) of the 20 mg PIROXEN Tablet solution were taken as prepared “Piroxicam Tablet Formulation 20mg PIROXEN “

to obtain concentrations (7.5,15,20 µg/mL) in a 20 mL volumetric flask. They were treated according to the optimal working method, then the absorbance (average of six readings) of each solution was measured against the sample

solution at wavelength 544 and the recovery and relative standard deviation were calculated. The results are presented in Table 10.

Table 10. Result of the method: accuracy and precision.

| Taken $\mu\text{g/mL}$ | Found $\mu\text{g/mL}$ | %,RE | %,Recovery | Average Recovery, % | %,RSD |
|------------------------|------------------------|-------|------------|---------------------|-------|
| 7.5 | 7.4 | 0.84 | 99.1 | 100.5 | 2.02 |
| 15 | 15.3 | -2.35 | 102.3 | | 0.38 |
| 20 | 20.02 | -0.13 | 100.1 | | 0.43 |

Standard Addition method

This study aimed to validate the specificity, precision, and accuracy of the proposed analytical method using a standard addition approach. The experimental design involved spiking fixed volumes (1–2 mL) of a pharmaceutical preparation (100 $\mu\text{g/mL}$) with increasing concentrations (0–2 $\mu\text{g/mL}$) of a standard piroxicam solution (100 $\mu\text{g/mL}$) in 20 mL volumetric flasks. All samples were processed following the same protocol used for calibration curve construction, with absorbance measurements taken at 544 nm against

a blank.

The results, detailed in **Table 11** and **Figure 7**, confirmed the method's reliability, demonstrating:

- **Specificity:** No interference from excipients in the pharmaceutical matrix
- **Accuracy:** High recovery rates (98–102%)
- **Precision:** Low variability (RSD < 2%)

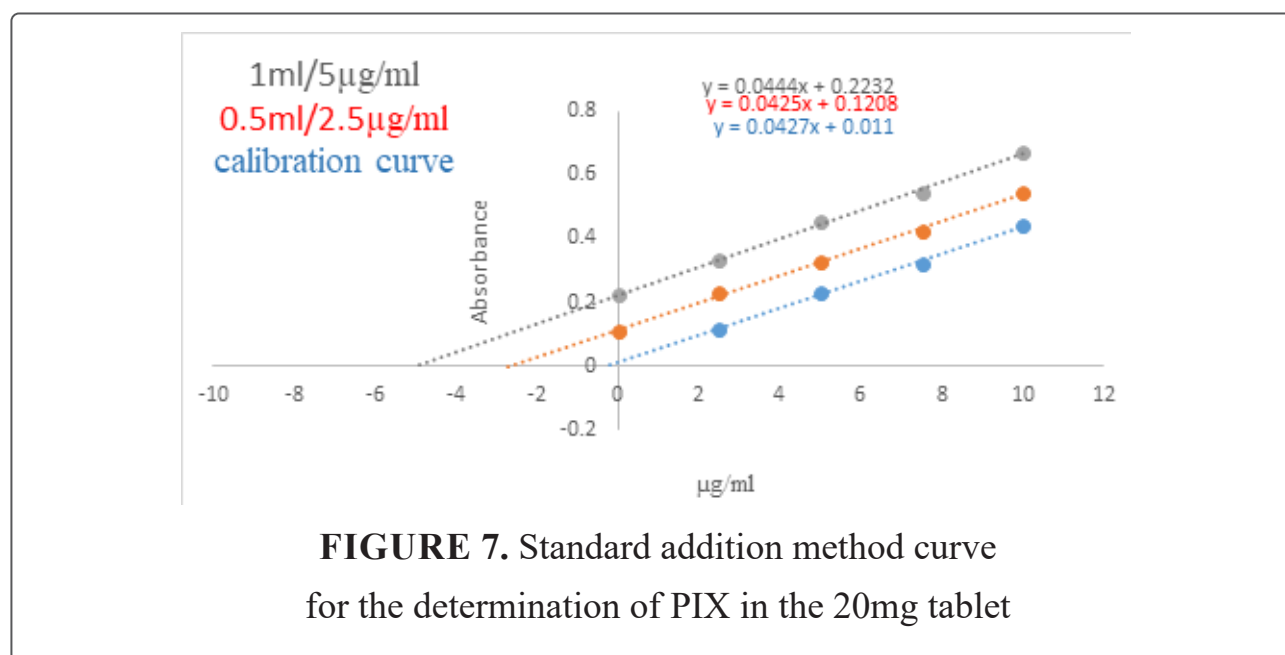
This validation aligns with ICH Q2 (R1) guidelines, ensuring the method's suitability for quality control applications.

Table 11. Result of the method: standard addition.

| Type of Drug | Piroxicam present $\mu\text{g/mL}$ | Piroxicam measured $\mu\text{g/mL}$ | %,Recovery |
|-----------------------|------------------------------------|-------------------------------------|------------|
| Tablet PIROX-EN 20 mg | 2.5 | 2.6 | 103 |
| | 5 | 4.77 | 95.5 |

The data shown in Table 17 shows that the standard addition method agrees closely with the direct method and is within an acceptable error range.

This consistency indicates that the standard addition method is reliable and free from interference.



CONCLUSION

A robust and accurate spectroscopic method for piroxicam has been developed, given its importance in pharmaceutical analysis. This method features stability, high sensitivity, and the absence of organic solvents or surfactants to avoid extraction, thus improving quality assurance and control. This method not only supports the increasing analytical requirements of the pharmaceutical industry but also provides laboratories with a reliable tool to ensure drug safety and efficacy.

References

1. Martindale, W. (2021). *The Complete Drug Reference*. Pharmaceutical Press.
2. Doloking, H., & Dhuha, N. S. (2024). Formation, Characterization, and In Vitro Dissolution Studies of Piroxicam-Malic Acid Cocrystals. *Research Journal of Pharmacy and Technology*, 17(7), 3061-3066
3. Mbah, C., Ogbonna, J., Nzekwe, I., Ugwu, G., Ezeh, R., Builders, P., & Ofoefule, S. (2021). Nanovesicle formulation enhances the anti-in-

- inflammatory properties and ensures the safe use of piroxicam. *Pharmaceutical Nanotechnology*, 9(3), 177-190.
- Hani, U., Osmani, R. A. M., Alqahani, A., Ghazwani, M., Rahamathulla, M., Almordy, S. A., & Alsaleh, H. A. (2021). 2 3 Full factorial design for formulation and evaluation of floating oral in situ gelling system of piroxicam. *Journal of Pharmaceutical Innovation*, 16, 528-536.
 - Banapura, A., & KR, M. (2024). A comparative evaluation of efficacy and safety of the combination of piroxicam with tramadol versus pentazocine in post-cesarean pain management. *National Journal of Physiology, Pharmacy & Pharmacology*, 14(10).
 - Batur, P. (2023). Adding piroxicam to levonorgestrel increased emergency contraception Impactive-ness—*Annals of Internal Medicine*, 176(12).
 - Sahu, C. R. (2016). Mechanisms Involved in Toxicity of Liver Caused by Piroxicam in Mice and Protective Impacts of Leaf Extract of *Hibiscus rosa-sinensis* L. *Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders*, 9.
 - Grosser, T., Smyth, E., & FitzGerald, G. A. (2011). Anti-inflammatory, antipyretic, and analgesic agents; pharmacotherapy of gout. *Goodman and Gilman's the pharmacological basis of therapeutics*, 12, 959-1004.
 - Dilleban, J., & Sundaresan, T. (2023). Role of non-steroidal anti-inflammatory drugs in orthopedics. *Sys Rev Pharm*, 14, 375-9.
 - Mostafa, G. A., Al-Dosseri, A. S., & Al-Badr, A. A. (2020). Piroxicam. In *Profiles of Drug Substances, Excipients and Related Methodology* (Vol. 45, pp. 199-474). Academic Press
 - Joint Formulary Committee. (2020). *British National Formulary 85*. BMJ Group and Pharmaceutical Press. London.
 - C. Martindale, *The Complete Drug Reference*, edited by S. C. Sweetman, 36th ed. (Pharmaceutical Press, London, 2009).
 - Ravisankar, P., Gowthami, S., & Rao, G. D. (2014). A review on an-

- alytical method development. *Indian journal of research in pharmacy and biotechnology*, 2(3), 1183
14. Siddiqui, M. R., AlOthman, Z. A., & Rahman, N. (2017). Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of Chemistry*, 10, S1409-S1421.
 15. Ahmed, S., Islam, S., Ullah, B., Biswas, S. K., Azad, A. S., & Hosain, S. (2020). A Review Article on Pharmaceutical Analysis of Pharmaceutical Industry According to Pharmacopoeias. *Oriental Journal of Chemistry*, 36(1).
 16. Soni, S., Ram, V., & Verma, A. (2018). Analytical method development and validation of piroxicam by high-performance liquid chromatography and ultraviolet spectroscopy technique. *Asian Journal of Pharmaceutical and Health Sciences*, 8(1).
 17. Caet, M. P., Monsores, M. A., Machado, A. K., Barth, T., Sangoi, M. S., & Todeschini, V. (2020). Pharmacopoeial HPLC methodology improvement: A case study of piroxicam. *Drug Analytical Research*, 4(2), 50-57.
 18. Shahbaz, N., Iqbal, Z., Nasir, F., Khan, F. U., Hassan, A. M., & Khan, S. I. (2018). Simultaneous determination of piroxicam and 5-hydroxy piroxicam: HPLC/UV method development, validation and application for pharmacokinetic evaluation in Pakistani population. *J Chem Soc Pak*, 40(05), 856-865.
 19. Abd El-Hay, S. S., El Sheikh, R., Gouda, A. A., Ali, M., & El-Sayed, H. M. (2022). Simultaneous estimation of pantoprazole and piroxicam by HPLC: Response surface methodology approach. *Microchemical Journal*, 176, 107247.
 20. Kaur, M., Mittal, S. K., & Chawla, R. (2022). Simultaneous estimation of tramadol and piroxicam by UV spectrophotometer and RP-HPLC. *Materials Today: Proceedings*, 48, 1735-1739.
 21. Chauhan, V., Grover, P., Bhardwaj, M., Kumar, S., & Nagarajan, K. (2024). Development and Validation of Fast and Sensitive RP-HPLC Stability-Indicating Method for Quantifying Piroxicam in Bulk Drug. *Journal of Chromatographic Science*.

22. Hou, W. R., & Lin, C. H. (2024, January). Capillary Electrophoresis Electrochemical Detection on a Thread-Based Microfluidic Platform with Penetrated Nanostructured Graphene Oxide Needles. In 2024 IEEE 37th International Conference on Micro Electro Mechanical Systems (MEMS) (pp. 232-235).
23. Dal, A. G., Oktayer, Z., & Doğrukol-Ak, D. (2014). Validated method for the determination of piroxicam by capillary zone electrophoresis and its application to Tablets. *Journal of Analytical Methods in Chemistry*, 2014(1), 352698,7.
24. Dal, A. G., Oktayer, Z., & Doğrukol-Ak, D. (2014). Validated method for the determination of piroxicam by capillary zone electrophoresis and its application to Tablets. *Journal of Analytical Methods in Chemistry*, 2014(1), 352698.
25. Ivanova, S., Todorova, V., Dyankov, S., & Ivanov, K. (2022). High-Performance Thin-Layer Chromatography (HPTLC) method for identification of meloxicam and piroxicam. *Processes*, 10(2), 394.
26. Puthli, S. P., & Vavia, P. R. (2000). Stability indicating HPTLC determination of piroxicam. *Journal of pharmaceutical and biomedical analysis*, 22(4), 673-677.
27. Starek, M., Krzek, J., & Rotkegel, P. (2015). TLC determination of piroxicam, tenoxicam, celecoxib and rofecoxib in biological material. *Journal of Analytical Chemistry*, 70, 351-359.
28. Zarei, E., Khaleghi, M. R., & Asghari, A. (2024). Development of ZnO-Pd/Bi₂O₃ nanocomposite-modified carbon paste electrode as a sensor for simultaneously determining piroxicam and naproxen. *Microchemical Journal*, 207, 111924.
29. Karimi-Maleh, H., Sheikhshoaei, I., & Samadzadeh, A. (2018). Simultaneous electrochemical determination of levodopa and piroxicam using a glassy carbon electrode modified with a ZnO-Pd/CNT nanocomposite. *RSC advances*, 8(47), 26707-26712. 40 .
30. de Oliveira, G. Z. D. M. G., Silva, F. W. L., Lopes, C. S. C., Braz, B. F., Santelli, R. E., & Cincotto, F.

- H. (2024). Development of a new highly sensitive electrochemical sensor to piroxicam anti-inflammatory determination using a disposable screen-printed electrode. *Ionics*, 30(5), 2793-2806.
31. Vu Ho, X. A., Dao, M. U., Le, T. H., Chuong Nguyen, T. H., Nguyen Dinh, M. T., Nguyen, Q. M., ... & Nguyen, C. C. (2023). Development of Electro-Reduced AgNPs/MnO₂/rGO Composite toward a Robust Sensor for the Simultaneous Determination of Piroxicam and Ofloxacin. *Industrial & Engineering Chemistry Research*, 62(11), 4778-4791.
32. Dhanalakshmi, N., Priya, T., Thenarasu, S., Sivanesan, S., & Thinnakaran, N. (2021). Synthesis and electrochemical properties of environmental free l-glutathione grafted graphene oxide/ZnO nanocomposite for highly selective piroxicam sensing. *Journal of Pharmaceutical Analysis*, 11(1), 48-56.
33. Ghobadpour, G., Farjami, F., & Fasihi, F. (2019). Sensitive electrochemical monitoring of piroxicam in pharmaceuticals using carbon ionic liquid electrode. *Current Pharmaceutical Analysis*, 15(1), 45-50.
34. Zhang, J. W., Li, R. F., Yao, L., Wang, Z. X., Lv, W. X., Kong, F. Y., & Wang, W. (2018). Highly sensitive determination of piroxicam using a glassy carbon electrode modified with silver nanoparticles dotted single-walled carbon nanotubes-reduced graphene oxide nanocomposite. *Journal of Electroanalytical Chemistry*, 823, 1-8.
35. Mahmood, R. M., Darweesh, S. A., Alassaf, N. A., & Al-Khalisy, R. S. (2024). Simultaneous Spectrophotometric Determination of Piroxicam, Naproxen, Diclofenac Sodium and Mefenamic Acid in Pharmaceutical Formulations by Partial Least Squares Method. *Methods & Objects of Chemical Analysis/Metody & Obekty Himičeskogo Analiza*, 19(2), 101 .
36. Sversut, R. A., Vieira, J. C., Rosa, A. M., do Amaral, M. S., Kassab, N. M., & Salgado, H. R. N. (2020). Validated spectrophotometric methods for simultaneous determination of oxytetracycline associated with diclofenac sodium or piroxicam in

- veterinary pharmaceutical dosage form. *Arabian Journal of Chemistry*, 13(1), 3159-3171
37. Nagaralli, B. S., Seetharamappa, J., & Melwanki, M. B. (2002). Sensitive spectrophotometric methods for the determination of amoxicillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations. *Journal of pharmaceutical and biomedical analysis*, 29(5), 859-864.
38. Gowda, B. G., Seetharamappa, J., & Melwanki, M. B. (2002). Indirect spectrophotometric determination of propranolol hydrochloride and piroxicam in pure and pharmaceutical formulations. *Analytical sciences*, 18(6), 671-674.
39. Amin, A. S. (2002). Spectrophotometric determination of piroxicam and tenoxicam in pharmaceutical formulations using alizarin. *Journal of pharmaceutical and biomedical analysis*, 29(4), 729-736.
40. Chamjangali, M. A., Bagherian, G., & Mehrjoo-Irani, S. (2012). Determination of Piroxicam in Different Pharmaceutical Products by a Simple Kinetic Procedure Based on An Induction Period Impact. *Analytical Chemistry Letters*, 2(1), 44-55
41. D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control* (Springer, Dordrecht, Netherlands, 1974).
42. El-Ries, M. A., Mohamed, G., Khalil, S., & El-Shall, M. (2003). Spectrophotometric and potentiometric determination of piroxicam and tenoxicam in pharmaceutical preparations. *Chemical and pharmaceutical bulletin*, 51(1), 6-10.
43. Stancheva, K., & Pasha, C. (2016). Spectrophotometric determinations of trace amounts arsenic (iii) and arsenic (v) using safranin o and fuchsine as new reagents. *Journal Oxidation Communications*, 39(2), 1538-1546.

