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Development of a Colorimetric Method for Determination of Piroxicam in Pharmaceutical Formulation Based on Charge Transfer Complex Formation Using Picric Acid

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ABSTRACT

Background: Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) that is used to manage mild to moderate pain, arthritis, and other inflammatory conditions. There is a dearth of simple, cost-effective, and reliable colorimetric methods for the routine determination of piroxicam in pharmaceutical formulations. The study was aimed at developing a colorimetric method for quantifying piroxicam based on charge-transfer complexation between piroxicam and picric acid.
Methods: The method is based on the formation of a charge-transfer complex between piroxicam an electron donor and picric acid as the electron acceptor. The yellow coloured product formed was quantified at the absorption maximum of 420 nm. The effect of various experimental conditions (reaction time, solvent type and reagent concentration) on complex formation were also investigated. The method was validated as per ICH guidelines. The accuracy was evaluated using recovery studies while precision was evaluated on intra-day and inter-day basis. The limit of detection (LOD), limit of quantification (LOQ) and molar absorptivity were determined and the method was compared with the official titrimetric assay method for the drug.
Results: The optimized conditions for complex formation were found to involve the use of Dichloromethane as solvent, a reaction time of 10 minutes and 2.5 ml of 0.001% picric acid solution. Beer's law was obeyed in the concentration range of 20 – 100 µg/ml ($r^2 = 0.994$). The method showed good precision with inter-day precision in the range of 0.03 – 0.91 % RSD while intra-day precision was from 0.02 – 0.85 % RSD. A comparison of the proposed method with the official method revealed that they produce comparable analytical results.
Conclusion: The developed method was applied successfully to the analysis of piroxicam in capsule formulation with good accuracy and precision and without any interference from the excipients. As such, the newly developed method can serve as a useful technique for the quality assessment of piroxicam.

1. INTRODUCTION

Piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide[1]) (Figure 1) is a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of oxicams[2]. Piroxicam possesses antipyretic and analgesic activities[3] and is a useful anti-inflammatory agent for the management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis[4,5]. Furthermore, it is used to relieve acute pain in musculoskeletal disorders[6], acute gout and for the treatment of primary dysmenorrhea[7]. Piroxicam acts by inhibition of the cyclooxygenase enzyme which is responsible for the biosynthesis of endogenous

prostaglandins[8]. Its non-selective action leads to simultaneous inhibition of both the constitutive COX-1 and inducible (COX-2) enzymes thereby resulting in some of its adverse effects such as GI bleeding[9].

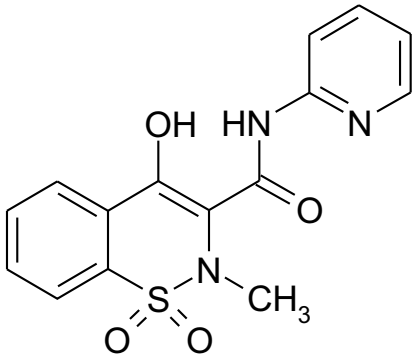

Figure 1: Structure of Piroxicam

The drug is official in several monographs such as the British Pharmacopoeia which describes a non-aqueous titrimetric method with 0.1 M perchloric acid for the drug[10] and the United States Pharmacopoeia[11] which describes a reverse-phase high performance liquid chromatographic (RP-HPLC) with UV detection method for determination of the drug. Some unofficial assay methods have similarly been developed for the analysis of piroxicam including; Thin Layer Chromatography-Mass spectrometry (TLC-MS)[12]; supercritical fluid chromatography-tandem mass spectrometry[13]; UV Spectrophotometry[14,15]; Spectrofluorimetry[16,17]; Chemiluminescence[18]; Capillary Zone Electrophoresis[19], micellar electro kinetic capillary chromatography[20]; HPLC-amperometry[21]; HPLC-UV method[22] and Electrochemical methods[23]. Some of these methods have demerits such as low sensitivity and requiring high concentration of reagents. In addition, some of these methods require the use of costly equipment, or utilize long analysis time hence may not be very suitable for routine analysis in the laboratory.

Colorimetric methods generally have some strengths such as acceptable sensitivity, reasonably short time of analysis, simplicity of operation and low analytical cost. Several colorimetric methods have been developed for piroxicam determination using different reagents such as sulphonphthalein acid dyes (bromothymol blue, bromophenol blue and bromocresol green)[24]; tetracyanoquinodimethane (TCNQ), tetracyanoethylene (TCNE)[25]. Picric acid has previously been used as a chromogenic reagent for the colorimetric determination of other drugs including Lansoprazole[26] and Aprepitant[27]. After an exhaustive literature search, it was observed that there is no previous study reporting the use of picric acid for the colorimetric determination of piroxicam in pharmaceutical formulations. The aim of this study therefore was to develop and validate a simple and cost effective colorimetric method for the analysis of piroxicam in pure and commercial dosage forms. The proposed method is based on the formation of charge transfer complexes between piroxicam and picric acid.

MATERIALS AND METHODS

Equipment

A UV-Vis double beam spectrophotometer (Model: UV-1650PC, Shimadzu, Japan) was used for all absorbance measurements, Analytical Weighing balance (Mettler Toledo, Switzerland), Vortex mixer (Milano-Italy) was also used.

Reagents and Chemicals

Five (5) different brands of piroxicam 20 mg capsules were purchased from retail pharmacy outlets in Jos, Nigeria and coded A to E. Piroxicam reference powder (99.99 % purity) was a gift from Prof. Ikoni Ogaji. Picric acid (Surechem, England), Methanol (Sigma-Aldrich, Germany), Dichloromethane (Lobachemie Co. Ltd, India), Diethyl ether (Riedel-de Haen, Germany), Chloroform (Lobachemie Co. Ltd, India).

Preparation of solutions and reagents

Piroxicam Standard Solution

Standard solution of piroxicam was prepared by weighing and dissolving 0.1 gram of piroxicam reference powder in about 20 mL of Methanol in a 100 mL volumetric flask and making up the volume to mark with Methanol. This solution was further diluted to give working standard solutions.

Picric acid Solution

Standard solution of picric acid was prepared by weighing and dissolving 0.001 gram of picric acid in about 30 mL of Methanol in a 100 mL volumetric flask and making up the volume to mark with the solvent.

Analytical Procedure

A 1 mL portion of piroxicam standard solution (20 µg/mL) was transferred into a 10 mL volumetric flask using a pipette. Then, 1 mL of picric acid solution was added and the volume made was up to mark with methanol. The resultant solution was mixed thoroughly using a vortex

mixer and the solution was allowed to react for 10 minutes before scanning in the visible region (350-700 nm) to determine the wavelength of maximum absorbance (λ_{max}). Absorbance readings were subsequently measured at the λ_{max} against a reagent blank. All preparations and measurements were done in triplicates.

Determination of Optimal Reaction Conditions for Complexation

Optimum reaction conditions for the formation of the charge transfer complex were investigated by determining the effects of reagent concentration, type of solvent used and reaction time on the formation of the charge transfer complex.

Effect of Reagent Concentration

The effect of picric acid concentration was evaluated by repeating the procedure and varying the volume of the picric acid solution. The volume of picric acid solution used include; 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL respectively. All solutions were prepared in triplicates and their corresponding absorbance was measured at the λ_{max} against a reagent blank. The mean absorbance was calculated.

Effect of Solvent type

The effect of solvent on complex formation was investigated by varying the organic solvents used in the reaction. Dichloromethane (CH_2Cl_2), Chloroform ($CHCl_3$), Methanol (CH_3OH) and Diethyl ether $(C_2H_5)_2O$ were evaluated in this experiment with absorbance measured at the λ_{max} against the suitable reagent blank. All solutions were prepared in triplicates and the mean absorbance was calculated.

Effect of Reaction Time

The effect of reaction time was investigated by varying the reaction time in 10-minute increments up to 50 minutes (i.e. 10, 20, 30, 40 and 50 minutes). Absorbance readings were taken in triplicates at λ_{max} against a reagent blank for each reaction time and the mean absorbance was calculated.

Stability of the Charge Transfer Complex

The stability of the charge transfer complex formed from the reaction of piroxicam with picric acid under optimized conditions was observed over a 24-hour period.

Stoichiometric Relationship of the Reaction

The stoichiometric relationship between piroxicam and picric acid was investigated using the Jobs method of continuous variation. In this procedure, equimolar concentrations of piroxicam and picric acid were prepared. A series of 10 mL volumetric flasks were set up and the prepared solution piroxicam and picric acid were added in the ratio; 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 respectively. The resultant solutions were mixed thoroughly using a vortex mixer, and allowed to react for 10 minutes, Afterwards, the absorbance was measured at the λ_{max} against a reagent blank.

Construction of Calibration Curve

A 100 mg in 100 mL (1 mg/mL) of piroxicam solution was prepared using methanol. Dilutions of strength 20, 40, 60, 80 and 100 µg/mL were prepared in triplicates. The dilutions were subjected to the analytical procedure and their corresponding absorbances were measured at the λ_{max} against a reagent blank. The mean absorbance was calculated and a graph of mean absorbance against concentration was plotted.

Method Validation

Determination of Accuracy

The accuracy of the method was evaluated through recovery experiments carried out by adding known amounts of standard solutions to commercial sample solutions[28]. The percentage recovery was calculated using the formula:

$\% \text{ Recovery} = [(C_F - C_U)/C_A] \times 100$

where C_F is the concentration of analyte measured in the fortified test sample and C_U is the concentration of analyte measured in the unfortified test sample while C_A represents the concentration of the analyte added to the fortified test sample

Determination of Precision

Intra-day and Inter-day experiments were used to evaluate the precision of the method. Triplicates of three different concentrations of piroxicam were prepared (i.e. 50, 100, 150 µg/mL) and these were analyzed three times on the same day to obtain the intraday readings. They were similarly analyzed three times daily for three consecutive days to obtain the inter-day precision which is represented by the % Relative Standard Deviation.

$\% \text{ Relative Standard Deviation (RSD)} = S \times (^{100}/x)$

S = Standard deviation

X = Mean absorbance

Determination of Limit of Detection and Limit of Quantification

Limit of Detection (LOD) was calculated using the formula $LOD = 3.3 \times (\sigma/S)$ while Limit of Quantification (LOQ) was determined using the formula, $LOQ = 10 \times (\sigma/S)$. Where,

σ = Standard deviation of intercepts of calibration curve

S = Slope of the regression line of the calibration curve

Determination of linearity and sensitivity

The linearity was determined from the correlation coefficient (r^2) while the sensitivity was determined from the slope of the regression line of the calibration curve.

Application of the developed method to the analysis of the tablet dosage formulation

The developed method was applied to five (5) different brands of piroxicam capsules obtained from different community pharmacies. In the procedure, the contents of 20 capsules of piroxicam were weighed from brand A (each capsule contains 20 mg of piroxicam). The powder was homogenized and a quantity of this powder equivalent to 20 mg piroxicam was weighed and diluted with solvent. A 50 µg/mL solution was prepared by dilution and 1 mL of the solution was then transferred into three (3) different volumetric flasks separately. To this, 2.5 mL of picric acid solution (0.001 % $^{w}/_{v}$) was added and the volume made up to the 10 mL mark. The resultant solution was mixed using a vortex mixer and allowed to react for 10 minutes. The absorbance was measured at the λ_{max} against a reagent blank. The procedure was repeated for brands B – E.

Official Titrimetric Assay[10]

The content of twenty (20) capsules of the brand were weighed individually and the average weight was calculated. The powder was homogenized and the weight equivalent to 0.250 g of piroxicam was accurately weighed and dissolved in a 60 mL mixture of equal volumes of Acetic Anhydride R and Anhydrous Acetic Acid R. The resultant solution was then titrated against 0.1 Molar Perchloric acid and the end point was determined using 2 drops of crystal violet indicator. The same procedure was repeated using the other four brands.

RESULTS

The wavelength of maximum absorption of the piroxicam-picric acid charge transfer complex was found to be 420 nm when the solution was scanned between 350 to 700 nm. The effect of varying the concentration of the derivatizing reagent (picric acid) on complex formation was investigated and it was found that the maximum absorbance of the piroxicam-picric acid charge transfer complex was obtained when 2.5 mL of the picric acid solution was used (Figure 2).

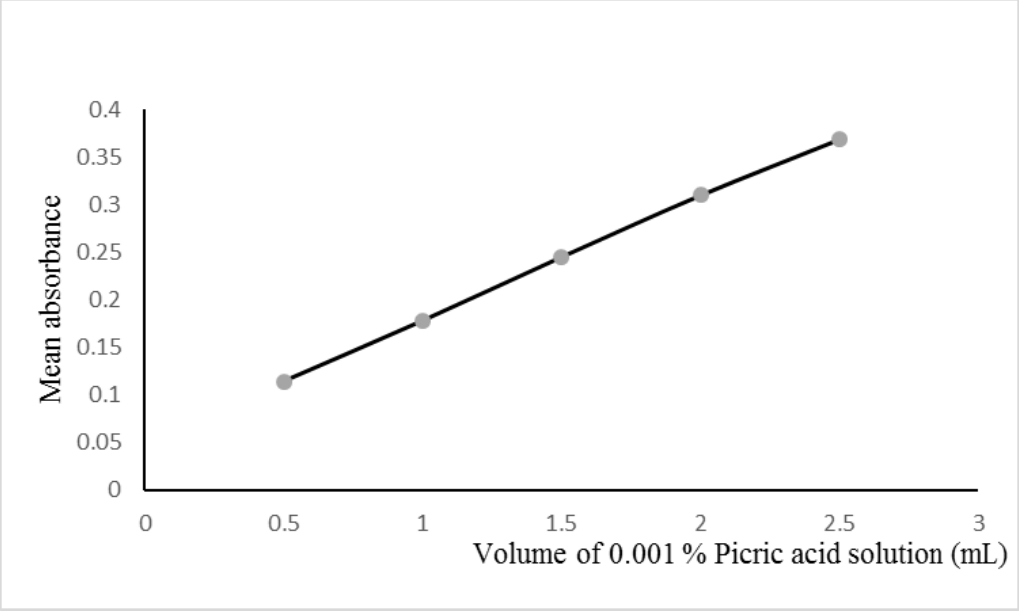

Figure 2: Effect of Reagent Volume on Piroxicam-Picric Acid Charge Transfer Complex

Figure 3 shows the effect of different solvents on complex formation and it can be seen that Dichloromethane was the best solvent for the formation of the piroxicam-picric acid complex.

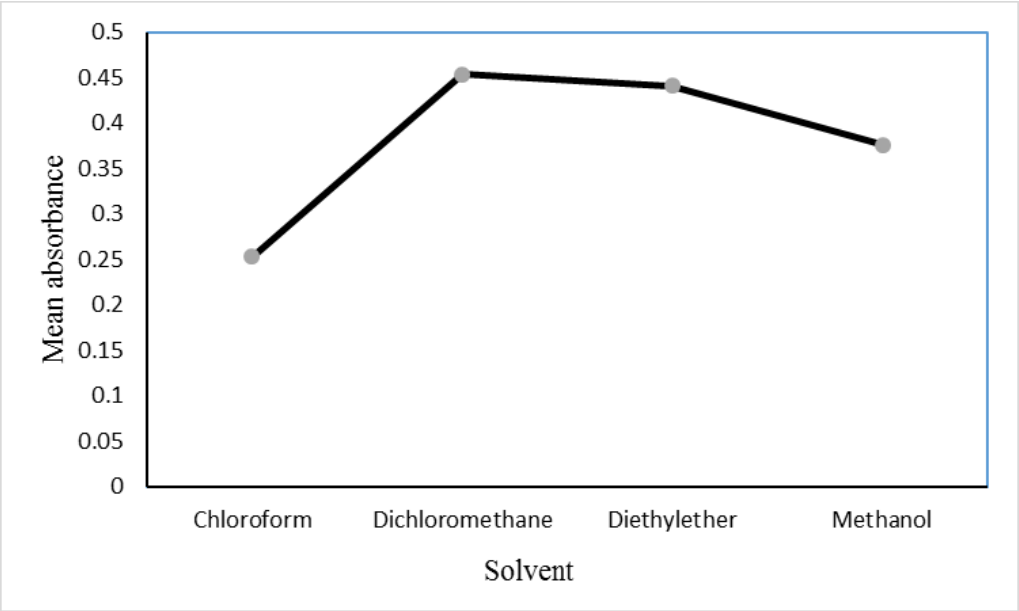

Figure 3: Effect of Solvent on Piroxicam-Picric Acid Charge Transfer Complex

The effect of reaction time on complex formation is depicted in Figure 4 and it can be seen that optimal complex formation occurred after 10 minutes of reaction.

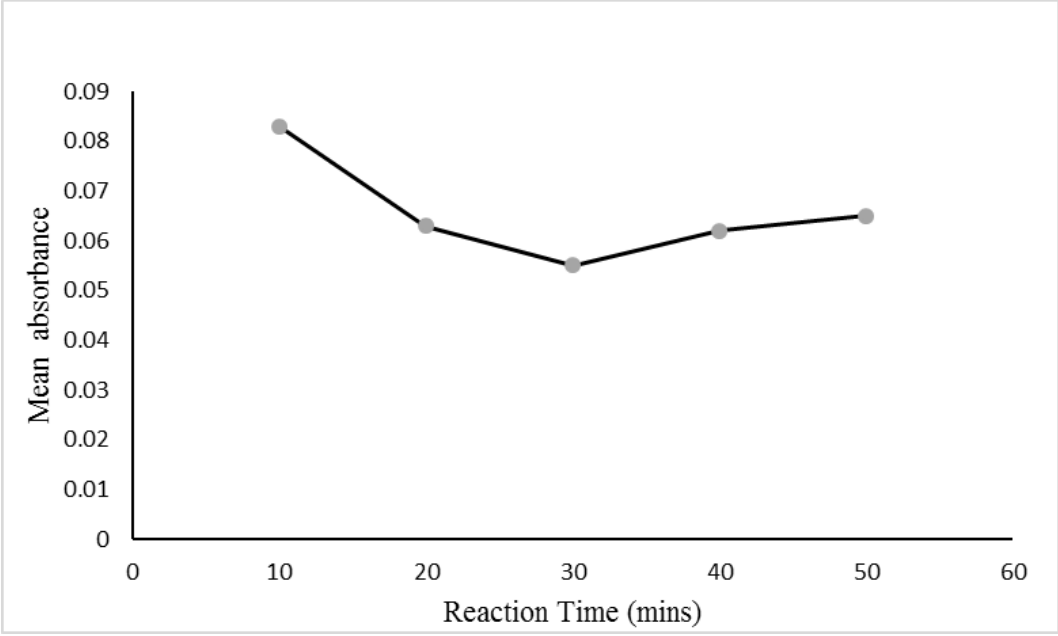

Figure 4: Effect of Reaction Time on Piroxicam-Picric Acid Charge Transfer Complex

The stability of the Piroxicam-Picric Acid Complex was investigated and it was found to remain stable for 24 hours after formation (Figure 5).

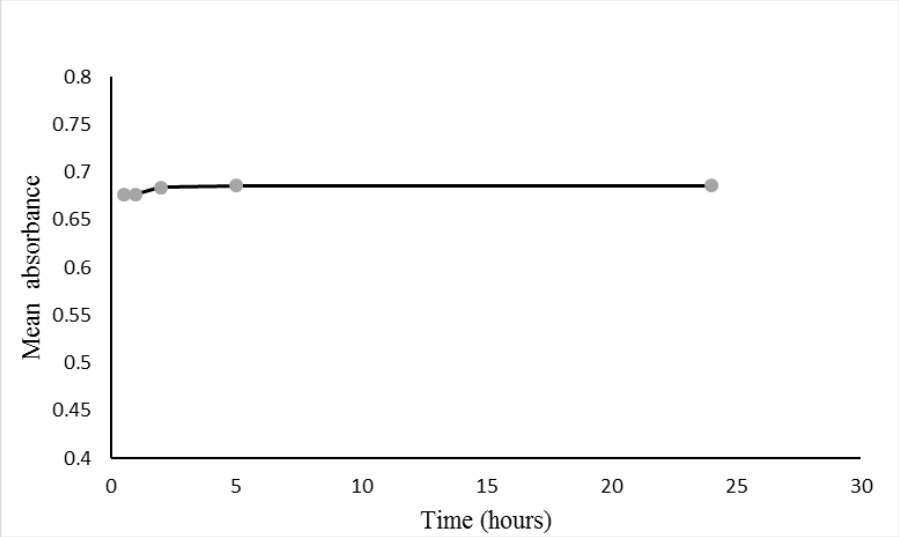

Figure 5: Stability of the Piroxicam-Picric Acid Charge Transfer Complex over 24 hours

Figure 6 shows the Jobs Plot of continuous variation which shows a 1:1 mole ratio for the reaction between Piroxicam and Picric Acid.

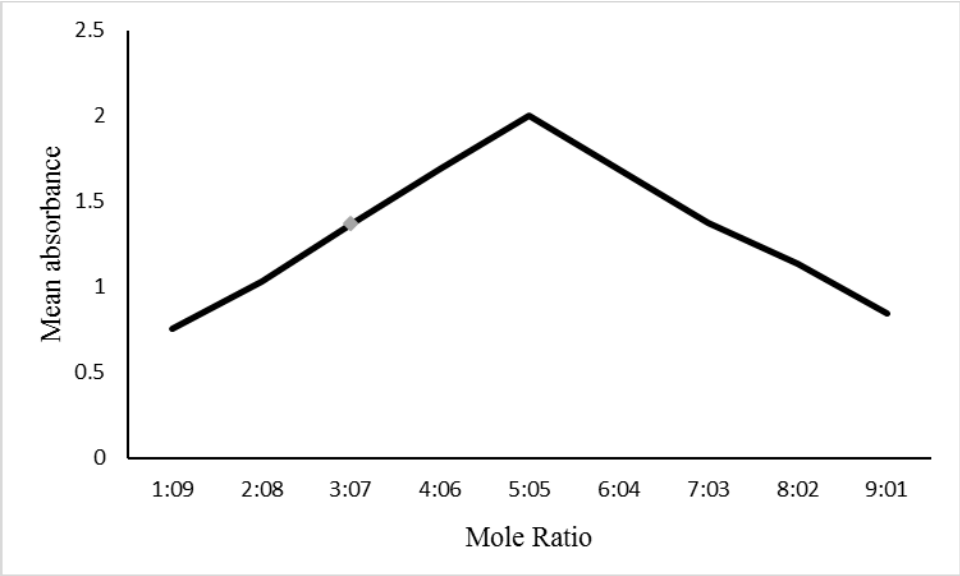

Figure 6: Continuous Variation Plot Showing Stoichiometric relationship between Piroxicam and Picric Acid in the complex formation.

Figure 7 shows the calibration curve of Absorbance versus Concentration for the Piroxicam-Picric Acid Charge Transfer Complex.

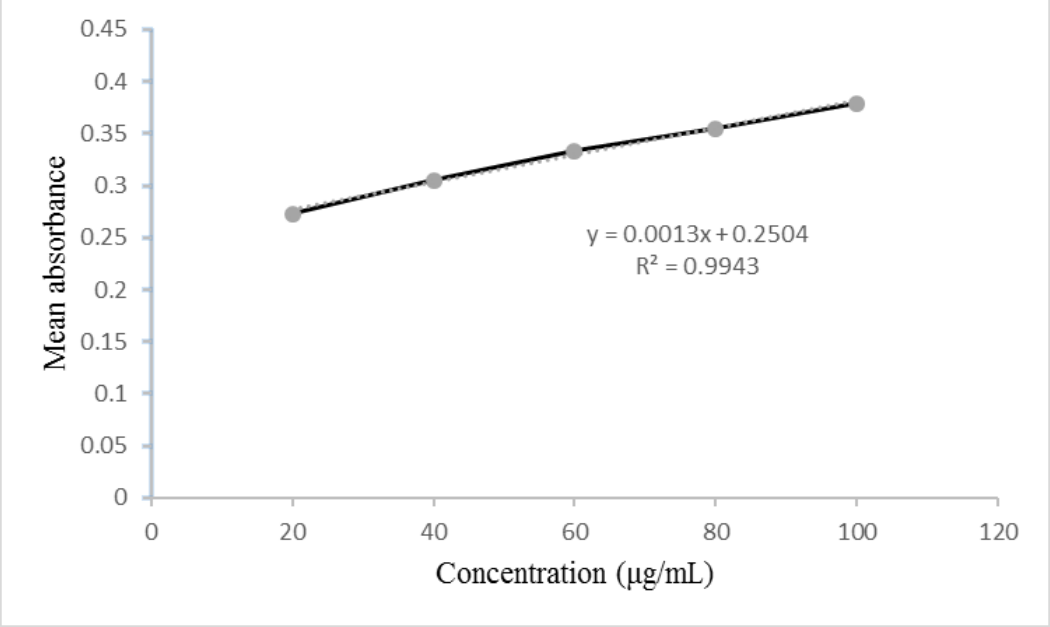

Figure 7: Calibration curve for Piroxicam-Picric Acid Charge Transfer Complex

Table 1 shows the percentage content of various brands of 20 mg Piroxicam capsules determined using both the developed charge transfer complexation method and the official titrimetric assay method. Table 2 shows the summary of the analytical performance characteristics for the newly developed method.

Table 1: Percentage Content of Piroxicam Capsule Brands by the Developed and Official Titrimetric Method

Brand Code	% content by Developed Method	% content by the Official Titrimetric Method (British Pharmacopoeia)[10]
A	100.07 ± 1.42	101.03 ± 0.51
B	101.34 ± 0.79	100.05 ± 0.82
C	93.99 ± 1.01	95.00 ± 0.44
D	98.70 ± 1.03	99.70 ± 0.98
E	103.89 ± 1.77	104.03 ± 1.06

Table 2: Summary of Analytical Performance Parameters for the Piroxicam-picric acid charge transfer complexation method

S/N	Parameter	Values
1.	Wavelength of Absorption Maximum (λ_{max})	420 nm
2.	Regression equation	Yabs = 0.001x + 0.250
3.	Correlation coefficient (r^2)	0.994
4.	Linear range (µg/mL)	20- 100
5.	Accuracy (Mean % Recovery)	100.56 ± 0.45
6.	Precision (% RSD)	
	Intra - day precision	0.02- 0.85
	Inter - day precision	0.03- 0.91
7.	Limit of Detection (µg/mL)	0.68
8.	Limit of Quantitation (µg/mL)	1.37
9.	Molar Absorptivity ($LMol^{-1}cm^{-1}$)	4,522.93

DISCUSSION

Charge transfer interactions have been shown to produce slight modifications in the properties (both chemical and physical) of the unbound donor and the acceptor molecules which leads to a change in colour[29]. Based on this, several charge transfer methods have been developed for the efficient quantitative determination of pharmaceuticals[30]. These methods are very reliable and accurate as compared to other analytical methods for pharmaceutical determination.

In this study, piroxicam which contains several basic Nitrogen atoms that can serve as n-donor, was used with picric acid (2,4,6-trinitrophenol) as a π-acceptor. The interaction of the two in dichloromethane formed a strong and stable complex and this was accompanied by a perceptible change in colour. The deep yellow-coloured charge-transfer complex absorbed maximally at 420 nm. The interaction between picric acid (the π-acceptor) and piroxicam (the n-donor) is a charge-transfer complexation reaction due to the transfer of a proton (H^+) from the -OH group of picric acid to the nitrogen atom in the pyridine ring of piroxicam. Picric acid is colourless when dissolved in dichloromethane but becomes yellow when piroxicam is added as a result of the formation of the phenolate (picrate) anion[26]. The likely pathway for the complexation reaction is shown in Figure 8.

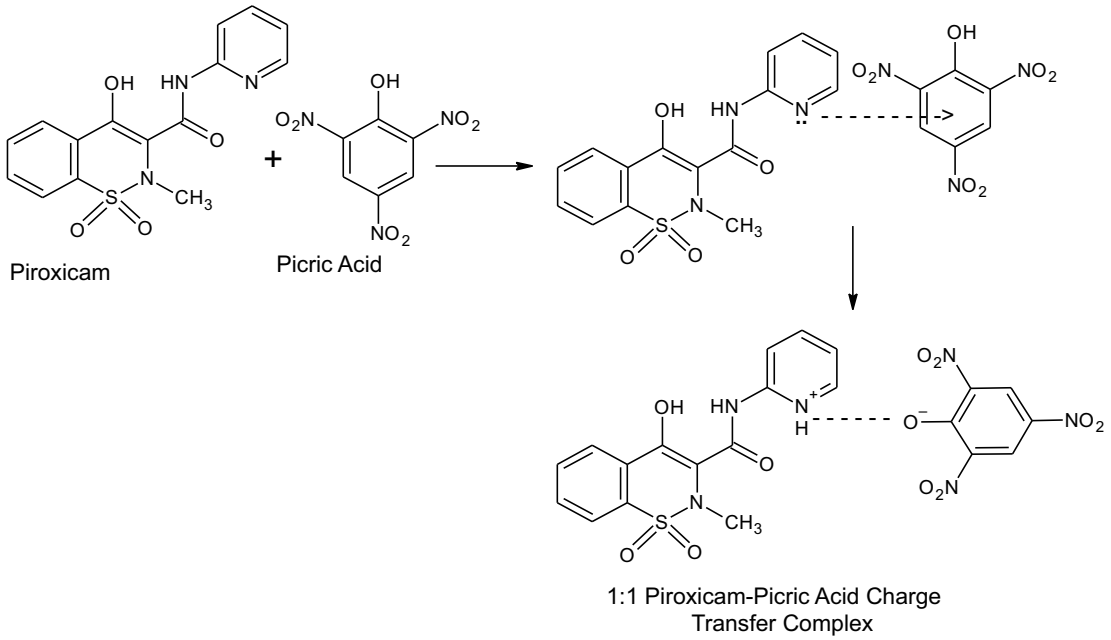

Figure 8: Proposed reaction pathway for formation of Piroxicam-Picric Acid charge-transfer complex.

Optimization of reaction conditions

The effect of reagent concentration on the absorption intensity at 420 nm was evaluated using different volumes of picric acid. In this method, 2.5 ml of 0.001% picric acid was found to provide maximum absorbance (Figure 6) and this was subsequently used throughout the experiment. In order to choose an appropriate solvent for the charge transfer complexation, the reaction of piroxicam with picric acid was studied in several solvents of varying polarity including dichloromethane (dielectric constant: 8.93), chloroform (dielectric constant: 4.80), methanol (dielectric constant: 32.70) and diethylether (dielectric constant: 4.30). Solvent polarity is known to influence the dynamics of charge transfer complexation and in this study, Dichloromethane which is a solvent of intermediate polarity was found to be superior over other solvents in terms of stability of the complex and sensitivity. This contrasts with another study where the charge transfer complexation of piroxicam with tetracyanoethylene and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone was best in Acetonitrile and Methanol which are polar solvent[25]. The optimal reaction time for colour development at room temperature (25 ± 2 °C) was evaluated, and it was observed that complete colour development was attained after 10 minutes of reaction. The coloured complex formed was stable for over 24 hours which is longer than the 3 hour

stability exhibited by the charge transfer complex formed between piroxicam and tetracyanoethylene in another study[25]. In assessing the stoichiometry of the interaction between picric acid and piroxicam using Jobs plot, maximum absorbance was obtained when equal amounts of equimolar solutions of picric acid and piroxicam (Figure 6) were used indicating that a 1:1 complex is formed between the two interacting compounds.

Method validation

After optimizing the experimental conditions, the absorbance readings were found to be linear relative to piroxicam concentration over the range of 20 to 100 µg/ml (r = 0.994). The developed method has a broader linear concentration range compared to previous studies[30] in which colorimetric methods for piroxicam were developed involving its oxidation with ceric ammonium sulfate in an acidic medium which had a narrow linear concentration range of 0.2 - 10 µg/mL[31] and another study[32] which utilized reaction of piroxicam with copper sulfate pentahydrate to yield yellow coloured products that were analyzed colorimetrically within a linear range of 2-12 µg /mL. The accuracy of the proposed method was found to be good with a mean value of 100.56 ± 0.45% obtained in the recovery experiment which falls within the acceptable range of 95-105 % as specified in the FDA guidance on method validation[33]. This value is comparable with the 100.3 ± 0.8% value obtained in another method development study based on Iron (III) mediated oxidation of piroxicam in the presence of o-phenanthroline to form a feroin complex[34]. The developed method exhibited low values of relative standard deviation (RSD: 0.02 - 0.85 % for intra-day) and (RSD: 0.03 - 0.91% for inter-day) which are suggestive of the method's high precision as the values all fall below the ICH[35] threshold of RSD ≤ 2%. These values also signify that the method is more precise than an ion pair complexation method developed for piroxicam via its reaction with various Alizarin derivatives where relative standard deviation value of 1.2 % was obtained[36]. Furthermore, the values of LOD (0.68 µg /mL) and LOQ (1.37 µg /mL) indicate that the new method has adequate sensitivity for the determination of piroxicam in pharmaceutical products. The method was found to be more sensitive than a previously developed method for the drug which had LOD and LOQ of 0.92 and 3.0 µg /mL respectively[25].

Application of Developed Method to Analysis of Piroxicam Capsule Formulation

The developed charge transfer method was successfully applied for the quantification of piroxicam in five different brands of commercial capsules. The results obtained by the proposed method were compared to those of the reference titrimetric method in which piroxicam was titrated against 0.1 M NaOH. The results presented in Table 1 confirm that there is good concordance between the analytical results obtained using the official reference method and the developed method.

CONCLUSION

A simple and rapid colorimetric method for the determination of piroxicam in capsule formulation was developed and validated following ICH guidelines. The method is based on the well-established charge-transfer complexation reaction involving picric acid as an analytical reagent. In comparison with most of the existing methods for the determination of piroxicam, the newly developed method is very simple and cost-effective as it involves simply mixing the drug and picric acid in dichloromethane and measuring the absorbance. Limited selectivity of the method for the analyte is recognized as a weakness of the method. However, it has the advantages of not requiring any complex extraction steps and its effectiveness at ambient temperature. These attributes together with its high sensitivity and the use of simple and inexpensive equipment, suggest that this method can be used in routine quality evaluation of piroxicam tablets.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Sadeghi M and Shabani-Nooshabadi M (2021) High sensitive titanium/chitosan-coated nanoporous gold film electrode for electrochemical determination of acetaminophen in the presence of piroxicam. *Progress in Organic Coatings*, 151: 106100 https://www.sciencedirect.com/science/article/pii/S0300944020313114
2. Mishra M, Chawla V and Chawla P (2019) Pectin, beta-cyclodextrin, chitosan and albumin based gastroprotective systems for piroxicam maleate: Synthesis, characterization and biological evaluation. *International Journal of Biological Macromolecules,* 122: 127–36 https://www.sciencedirect.com/science/article/pii/

S0141813018350220

3. Raja MU, Tauchman J, Therrien B, Süss-Fink G, Riedel T and Dyson PJ (2014) Arene ruthenium and pentamethylcyclopentadienyl rhodium and iridium complexes containing N,O-chelating ligands derived from piroxicam: Synthesis, molecular structure and cytotoxicity. *Inorganica Chimica Acta*, 409: 479–83. https://www.sciencedirect.com/science/article/pii/S0020169313004611
4. Abdeen A, Aboubakr M, Elgazzar D, Abdo M, Abdelkader A, Ibrahim S and Elkomy A (2019) Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats. *Biomedicine and Pharmacotherapy*, 110: 895–905. https://www.sciencedirect.com/science/article/pii/S075333221837080X
5. Samra MM, Hafeez H, Azam M, Imran M and Basra MAR (2023) Bi(III) complexes of piroxicam and meloxicam: Synthesis, characterization, antioxidant, anti-inflammatory and DNA cleavage studies. *Journal of Molecular Structure*, 1272: 134234. https://www.sciencedirect.com/science/article/pii/S0022286022018853
6. Salma H, Melha YM, Sonia L, Hamza H and Salim N (2021) Efficient Prediction of In Vitro Piroxicam Release and Diffusion From Topical Films Based on Biopolymers Using Deep Learning Models and Generative Adversarial Networks. *Journal of Pharmaceutical Science,* 110 (6): 2531–43. https://www.sciencedirect.com/science/article/pii/S0022354921000745
7. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, and Bolton EE (2019) Piroxicam | C15H13N3O4S – PubChem 2019 update: improved access to chemical data. https://pubmed.ncbi.nlm.nih.gov/30371825/
8. Ong SM, Saeki K, Tanaka Y, Nishimura R and Nakagawa T (2016) Effects of etoposide alone and in combination with piroxicam on canine osteosarcoma cell lines. *The Veterinary Journal*, 218: 51–9. https://www.sciencedirect.com/science/article/pii/S1090023316301927
9. Szczęśniak-Sięga BM, Mogilski S, Wiglusz RJ, Janczak J, Maniewska J, Malinka W and Filipek B (2019) Synthesis and pharmacological evaluation of novel arylpiperazine oxicams derivatives as potent analgesics without ulcerogenicity. *Bioorganic and Medicinal Chemistry*, 27 (8): 1619–28. https://www.sciencedirect.com/science/article/pii/S0968089618316018
10. British Pharmacopoeia (2009) Monographs. The British Pharmacopoeia Commission. London: The Pharmaceutical Press, Her Majestys Stationary Office. Volume 1, p 586.
11. USP (2019) The United State Pharmacopeia. Rockville (MD): The United States Pharmacopeial Convention.
12. Crecelius A, Clench MR, Richards DS and Parr V (2004) Quantitative determination of Piroxicam by TLC-MALDI TOF MS. *Journal of Pharmaceutical and Biomedical Analysis*, 35 (1): 31–9.
13. Li X, Gao Y, Liu J, Zhang G and Zhang T (2018) A rapid analysis of piroxicam in beagle plasma applying evaporation-free liquid-liquid extraction by supercritical fluid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, 1100–1101: 93–9 https://www.sciencedirect.com/science/article/pii/S157002321830998X
14. Kormosh ZA, Hunka IP and Bazel YR (2011) Spectrophotometric determination of piroxicam. *Journal of Analytical Chemistry*, 66 (4): 378–83.
15. Basan H, Göğer NG, Ertaş N and Orbey MT (2001) Quantitative determination of piroxicam in a new formulation (piroxicam–β-cyclodextrin) by derivative UV spectrophotometric method and HPLC. *Journal of Pharmaceutical and Biomedical Analysis,* 26 (2): 171–8. https://www.sciencedirect.com/science/article/pii/S0731708501003831
16. Escandar GM, Bystol AJ and Campiglia AD (2002) Spectrofluorimetric method for the determination of piroxicam and pyridoxine. *Analytica Chimica Acta*, 466 (2): 275–83.
17. Zhang J, Li Q, Liu Z and Zhao L (2023) Rapid and sensitive determination of Piroxicam by N-doped carbon dots prepared by plant soot. Spectrochimica *Acta Part A*: *Molecular and Biomolecular Spectroscopy*, 299: 122833. https://www.sciencedirect.com/science/article/pii

/S1386142523005188
18. Pulgarín JAM, Molina AA and Boras N (2010) Determination of piroxicam in pharmaceutical preparations by continuous-flow chemiluminescence. *Analytical Methods*, 2 (1): 76–81.
19. Dal AG, Oktayer Z, Doğrukol-Ak D. Validated method for the determination of piroxicam by capillary zone electrophoresis and its application to tablets. *Journal of Analytical Methods in Chemistry*, 2014;2014:352698.
20. Donato MG, Baeyens W, Van Den Bossche W and Sandra P (1994) The determination of non-steroidal antiinflammatory drugs in pharmaceuticals by capillary zone electrophoresis and micellar electrokinetic capillary chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 12 (1): 21–6.
21. De Jager AD, Ellis H, Hundt HKL, Swart KJ and Hundt AF (1999) High-performance liquid chromatographic determination with amperometric detection of piroxicam in human plasma and tissues. *Journal of Chromatography B: Biomedical Sciences and Applications*, 729 (1–2): 183–9
22. Dragomiroiu GTAB, Cimpoieşu A, Ginghină O, Baloescu C, Bârcă M, Popa DE, Ciobanu A and Anuţa V (2015) The development and validation of a rapid HPLC method for determination of piroxicam. *Farmacia*, 63 (1): 123–31
23. Kong FY, Li RF, Yao L, Wang ZX, Lv WX and Wang W (2019) Pt nanoparticles supported on nitrogen doped reduced graphene oxide-single wall carbon nanotubes as a novel platform for highly sensitive electrochemical sensing of piroxicam. *Journal of Electroanalytical Chemistry*, 832: 385–91. https://www.sciencedirect.com/science/article/pii/S1572665718307598
24. Alizadeh N and Keyhanian F (2014) Sensitive and selective spectrophotometric assay of piroxicam in pure form, capsule and human blood serum samples via ion-pair complex formation. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 130: 238–44. https://www.sciencedirect.com/science/article/pii/S1386142514004855
25. Assubaie FN (2013) Utilization of charge transfer complex formation for the spectrophotometric determination of piroxicam and tenoxicam. *Analytical Chemistry: An Indian Journal*, 13 (1): 69–76
26. Abdulrahman SMA, Devi OZ, Basavaiah K and Vinay KB (2016) Use of picric acid and iodine as electron acceptors for spectrophotometric determination of lansoprazole through a charge-transfer complexation reaction. *Journal of Taibah University for Science*, 10 (1): 80-91
27. Chaitanya M, Kumar TH, Koduru S and Kalepu S (2025) Quantification of Aprepitant Via Charge Transfer Complexation Reactions Through Visible Spectrophotometric Methods. *Journal of Applied Spectroscopy*, 91: 1418–1427. https://doi.org/10.1007/s10812-025-01868-3
28. AOAC. (2005) Official Methods of Analysis. 18th Edition. Washington DC: Association of Official Analytical Chemists.
29. Adam AMA, Saad HA, Refat MS and Hegab MS (2022) Charge-transfer complexes of antipsychotic drug sulpiride with inorganic and organic acceptors generated through two different approaches: Spectral characterization. *Journal of Molecular Liquids*, 353: 118819. https://www.sciencedirect.com/science/article/pii/S0167732222003567
30. Haque SKM, Umar Y, Al-Batty S, Sarief A, Abu-Judeh A, Al-Awwad H and Rahman H (2023) Charge transfer based green spectrophotometric method to determine remogliflozin etabonate applying response surface methodology supported with computational studies in pharmaceutical formulations. *Sustainable Chemistry and Pharmacy*, 35: 101193
31. Gowda BG, Seetharamappa J and Melwanki MB (2002) Indirect Spectrophotometric Determination of Propranolol Hydrochloride and Piroxicam in Pure and Pharmaceutical Formulations. *Analytical Sciences,* 18 (6): 671–4. https://doi.org/10.2116/analsci.18.671
32. Okeri HA and Anthonia UO (2007) Colorimetric Analysis of Piroxicam. *Pakistan Journal of Scientific and Industrial Research*, 50 (1): 1–4
33. Food Drug Administrations (2020) Office of Regulatory Affair, ORA-LAB-Manual Volume II. ORALAB.5.4.5. Methods, Method Verification and Validation. https://www.fda.gov/media/73920/download
34. El-Didamony AM and Amin AS (2004)

Adaptation of a Color Reaction for Spectrophotometric Determination of Diclofenac Sodium and Piroxicam in Pure Form and in Pharmaceutical Formulations. *Analytical Letters*, 37 (6): 1151–62. https://doi.org/10.1081/AL-120034060

35. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005) (ICH) Q2(R1) Validation of Analytical Procedures: Text and Methodology.

36. Amin AS (2002) Spectrophotometric determination of piroxicam and tenoxicam in pharmaceutical formulations using alizarin. *Journal of Pharmaceutical and Biomedical Analysis*, 29 (4): 729–36. https://www.sciencedirect.com/science/article/pii/S0731708502000353