



Microdetermination of Piroxicam in Pharmaceutical Formulations by Complexation with Fe(III) and Image Scanning Densitometry

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Abstract: Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) that is used to relieve pain or inflammation due to osteoarthritis or rheumatoid arthritis. Assay of piroxicam in pharmaceutical formulations can be performed by using a number of analytical techniques. This work estimates the drug in commercial samples using a novel method, Computational Image Scanning Densitometry (CISD). Micro-volumes of the aqueous solution of piroxicam were reacted with iron(III) sulfate solution under optimum conditions on a white Teflon well plate to form a pink-colored mononuclear complex. By using a smartphone, the picture of the colored complex in the well plate was taken and transferred to an attached computer. The overall optical density resulting from red, green, and blue (RGB) components from a specific area of the colored image was measured and digitalized with the help of custom-made software. A standard curve was prepared by plotting optical density against piroxicam concentration. The method was simple, fast, adequately precise, and accurate for the assay of the drug in commercial samples. The validity of the new method was checked by comparing the results with those obtained by a standard spectrophotometric method of Piroxicam estimation.

Keywords: Piroxicam assay, Image scanning densitometry, Pharmaceutical analysis, Piroxicam estimation, Drug assay.

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1. INTRODUCTION

Piroxicam belongs to the oxycam class of nonsteroidal anti-inflammatory drugs (NSAIDs) usually employed to treat pain, fever, and inflammation in the body. Its structure is related to the enolic acid class of 4-hydroxy-1,2-benzothiazine carboxamides (1). As it belongs to a non-narcotic group of drugs, it can safely be used to relieve mild to moderate pains such as fractures, dental, postpartum, postoperative pain, arthritis, acute gout, and musculoskeletal disorders (2). Several merits of piroxicam, such as its long half-life, high efficiency, potency, and fewer side effects, have encouraged its use in various ailments (3). It is proven as a suitable alternative to diclofenac, aspirin, ibuprofen, phenylbutazone, indomethacin, ketoprofen, naproxen, and sulindac (4).

Besides numerous positive points, piroxicam may cause certain side effects. It involves about 83 destructive reactions, and 73 of these are related to the gastrointestinal tract such as peptic ulcer, hemorrhages, perforation, and bleeding (5). Therefore, an assay of piroxicam is important to achieve optimum therapeutic quantity and for a better quality of pharmaceutical preparations (6). Although several analytical techniques have been reported in the literature for the determination of piroxicam in pharmaceutical preparations such as HPLC (7,8), capillary electrophoresis (9), solid-phase extraction (10), flow injection method (11), voltammetry (12) potentiometric methods (13) and several electro-analytical and spectroscopic methods (6,14-20), yet most of them suffer from certain drawbacks and limitations, such as complicated procedures and sophisticated instrumentation for analysis. To overcome these problems, the present

work describes a novel method based on image scanning densitometry (ISD) for estimating piroxicam in commercial formulations.

During the last two decades, a number of workers have employed computers and smartphones for image scanning and developed new analytical methods (21-23). These methods are relatively fast, simple, environment-friendly, and cost-effective compared to classical spectrophotometric methods. Verma and his students used image processing to determine the turbidity in water samples (24). Lyra and colleagues assayed a number of drugs by using a digital image-based flame emission spectrometric method (25). The total amino acid contents of food samples have been determined by computational image scanning densitometry (26).

Computational Scanning Image Densitometry (CSID) is a new technique that our group developed for chemical analysis (27). CSID is as precise and accurate as spectrophotometry but faster and simpler and does not require a spectrophotometer. It is based on measuring the optical density of a colored spot resulting from the reaction of the analyte and the coloring reagent. Our group has employed this technique successfully for the quantification of several metal ions (28), sulfides (29), formaldehyde (30), mercury (31), arsenic (32), nickel and chromium (33). The present work is based on the complexation of piroxicam with Fe (III). Piroxicam forms a stable pink color complex with Fe (III), which absorbs maximum around 504 nm (6).

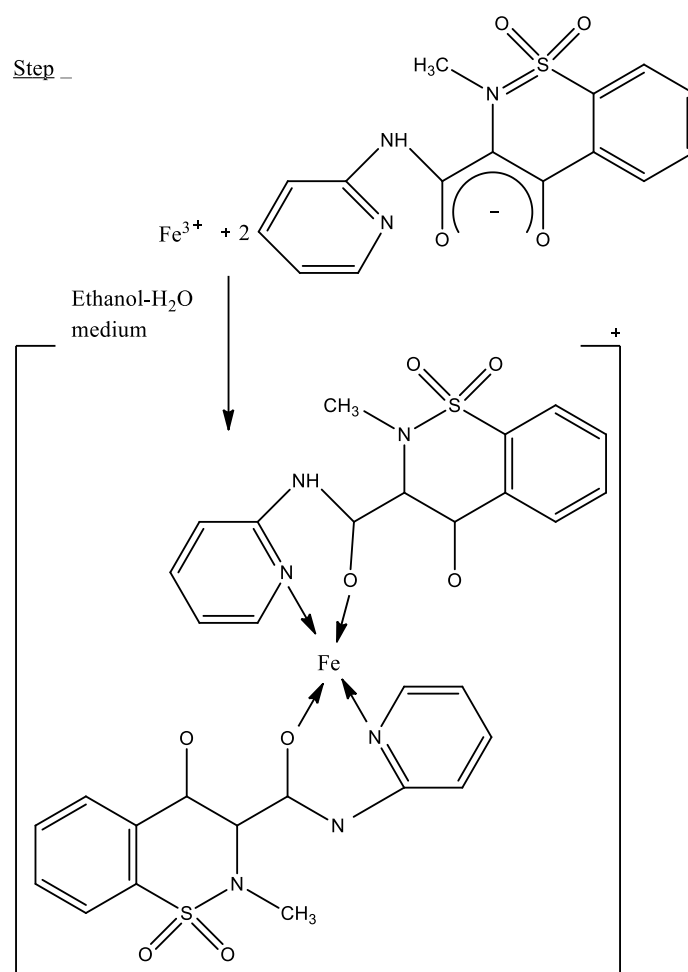


Figure 1: Chelation of Fe (III) with Piroxicam in water-ethanol media.

In the present work, micro-quantities of piroxicam in samples (in methanol-water) were mixed with a drop of iron (III) solution in a small well of Teflon well plate. A picture of colored solutions was taken with the help of a smartphone and transferred to an attached computer. Then, the optical density of each colored spot was measured with the help of customized software loaded on the computer. A calibration graph was prepared by plotting optical density against piroxicam concentration. To check the procedure's validity, a number of commercial samples containing piroxicam were analyzed using the proposed method and a standard spectrophotometric method, and the results obtained

from both methods were compared. The proposed method has proved itself fast, simple, accurate, and precise.

2. EXPERIMENTAL

2.1. Apparatus

For Image Scanning Densitometry, a 1 mL pipette (Pipetteman) and the well plate were used to develop colored spots. Cellphone cameras and ISD software (VB6-based graphical application with picture box, selection marquee, and flex grid) were used to measure the color density of digital images. A double-beam UV-visible spectrophotometer (Ultra-

3000 Rittun) with two quartz cells of 1 cm path length was used for spectrophotometric measurements.

2.2. Chemicals and Reagents

All reagents and organic solvents, including methanol and ethanol were of AnalaR Grade and were used in this work as such without further purification. Ferric sulfate (M.W. 399.88, Fluka Chemie AG, Switzerland) was purchased from the local market. Piroxicam reference standard drug (CAS No. 36322-90-4, M.W.= 331.4) was obtained from Sigma Chemical Company (St. Louis, USA) local agent. Capsules and tablets (Pfizer Ltd., Surrey, USA) of piroxicam were purchased from Scientific Pharmacy, Al-Khuwair, Muscat, Oman. For pH adjustment, Standard Buffers of pH 4, 7, and 10 were obtained from Hanna Instruments Inc. USA.

2.3. Preparation of Solutions

0.005 M Fe(III) solution was prepared by taking 0.2 g of $\text{Fe}_2(\text{SO}_4)_3$ in a 100 mL flask, and distilled water was used for the required dilution.

As Piroxicam was sparingly soluble in water hence, its 1000 ppm stock solution was prepared by dissolving 0.1 g of the drug in 10 mL of methanol and, after a little warming, filtered through the Whatman No. 42 filter paper. Then, the contents were diluted up to 100 mL with ethanol. Working solutions of piroxicam were prepared by diluting the 1000 ppm stock solution with ethanol.

Sample solutions of commercial dosage forms of Piroxicam (Feldene tablet, Pcam injection, Brexin tablet, Riacen capsule, Feldene capsules, Pcam tablet, Painza tablet, Pytex tablet, and Cyclodex tablet) were prepared in methanol. The powder contents of commercially available tablets and capsules (each 8 in number) of 10 mg strength of Piroxicam were taken in 25 mL methanol and, after a little warming, kept for 15 min for complete dissolution of the drug. The mixture was then filtered through Whatman No. 42 filter paper into 100 mL standard volumetric flasks. The residue was washed well with 4 to 5 mL portions of methanol for complete recovery of the drug, and each flask was diluted up to the mark with ethanol (as done for standards).

2.4. Optimization of Variables

All the possible variables, such as time, temperature, pH, volume of Fe(III) solution, and order of adding the reagents, were checked by reacting 5 mL of 1000 ppm solution of Piroxicam with different volumes of 0.005 M Fe(III) in a 100 mL flask under different experimental conditions. The volume was made up to the mark with ethanol. The absorbance of the final solution measured at 505 nm was taken as the measure of optimization.

2.4.1. Effect of concentration of Fe (III) solution

To check the effect of the concentrations of the Fe(III) solution, a 5 mL stock solution (1000 ppm) of piroxicam was made to react with different concentrations of a 0.005 M solution of Fe (III) in a series of 100 mL volumetric flasks. After 10 min, the red complex was diluted up to the mark with ethanol, and absorbance was measured against a

compensatory blank. The absorbance was plotted against the volume of the Fe(III) solution.

2.4.2. Effect of pH

To check the pH effect, the pH of the 5 mL solution of Piroxicam was adjusted to 4, 7, and 10 with the help of standard buffers in three 100 mL volumetric flasks. In each flask, 5 mL of 0.005 M Fe(III) solution was added, and after ten min, the volume was made up to the mark with ethanol. The absorbance of the red solution was measured against a compensatory blank.

2.4.3. Effect of time

To check the effect of time on the reaction of Piroxicam with Fe(III), 5 mL of stock solution (1000 ppm) of Piroxicam was reacted with 5 mL of 0.005M solution of Fe(III). After adjusting the pH at 4 of each solution, the contents were kept at room temperature for different intervals of time (5 to 50 min) before diluting the solution with ethanol and measuring the absorbance at 505 nm.

2.4.4. Effect of temperature

The effect of temperature was investigated by keeping the flasks at elevated temperatures (40, 60, and 80 °C) after adding 5 mL stock solution (1000 ppm) of Piroxicam with 5 mL of 0.005 M solution of Fe(III) in each flask. After heating for 10 min in a water bath, the contents of each flask were diluted up to the mark with ethanol, and the absorbance of each solution was measured at 505 nm against a compensatory blank.

2.4.5. Effect of order of reagents addition

To check the order of addition of reagents, the order between Piroxicam, Fe(III), and buffer was changed in the following sequence: a) Piroxicam + Fe(III) + pH-4 Buffer; b) Piroxicam + pH-4 Buffer + Fe(III); c) Fe(III) + pH-4 Buffer + Piroxicam. After mixing the reagents in different orders, the flasks were kept for 10 min and then diluted up to the mark with ethanol, and absorbance was measured at 505 nm.

2.4.6. Effect of organic solvents

To check the effect of various organic solvents, the contents of different flasks containing the reactants (Piroxicam + pH-4 Buffer + Fe (III)) were diluted with methanol, ethanol, acetone, ethyl acetate, and water after mixing them. In the case of dilution with water, the solution became significantly turbid.

2.5. Image Scanning Densitometric Method

Image Scanning Densitometry (ISD) was performed on a custom-made white Teflon plate with a series of wells of different capacities (0.25 to 1 mL). Piroxicam stock solution aliquots containing 10 to 100 ppm of the drug were transferred with the help of a micro-pipette into ten adjacent wells of 1 mL capacity into the plate. 0.2 mL buffer was added to each well, followed by 0.5 mL of 0.005 M solution of Fe(III). The contents were slightly mixed to allow the red color of varying intensity to develop in each well in the next ten min. Sample solutions of commercial formulations of Piroxicam were similarly treated in the next series of wells on the same Teflon plate. The image of the plate was captured with the help of a

smartphone or a camera and transferred to the computer, which was already loaded with custom-made image-scanning software. The color depth of each red-colored spot due to standard Piroxicam solution was measured with the help of the bespoke software (27). The calibration curve was obtained when the software plotted color intensity against Piroxicam concentration. The color intensity (optical density) of each spot of the commercial drug sample was also measured, and the quantity of the drug was calculated using the standard curve.

2.6. Reference (spectrophotometric) Method

Aliquots of 1000 ppm Piroxicam stock solution (1 to 10 mL) were transferred into ten clean and dry 100mL volumetric flasks. To adjust the pH at 4, 2 mL of standard pH 4 buffer was added to each flask. After thorough shaking, 5 mL of 0.005 M solution of Fe(III) was added to each flask and incubated for 10 min for complete complexation. The red complex was diluted up to the mark with ethanol. The absorbance of each homogeneous solution was measured against a compensatory blank at 505 nm. The Standard Curve was obtained by plotting the absorbance values against the concentration of Piroxicam. Then, the sample solutions of Piroxicam (pharmaceutical formulations) were treated similarly, and the absorbance of each solution was measured. The concentration of the drug in sample solutions was calculated from the Standard Curve.

3. RESULTS AND DISCUSSION

In this work, a novel method based on Image Scanning Densitometry (ISD) was developed for the assay of Piroxicam in commercial formulations. Our group has reported a number of techniques based on ISD (27-33). The method was optimized by analyzing the effect of different parameters such as solvent, pH, concentration of Fe(III), time, temperature and order of mixing the reagents and the results were compared with spectrophotometric method. The effect of all three colors (Red, Green Blue) of RGB channel were studied.

3.1. Effect of Organic Solvent

Piroxicam was sparingly soluble in water. Hence, some organic solvents had to be used to dissolve the drug. In addition, experimentation revealed that Piroxicam dissolved in methanol could not be diluted with water because the solution became significantly turbid and unfit for use in spectrophotometry. In this work, piroxicam solution prepared in methanol was diluted with ethanol, which gave the best results in terms of complete solubility and absorbance. In addition to ethanol, a few other polar solvents like acetone, methanol, and ethyl acetate were also employed for dilution. Still, as shown in Figure 2, maximum absorbance was obtained when ethanol was used as a diluting solvent.

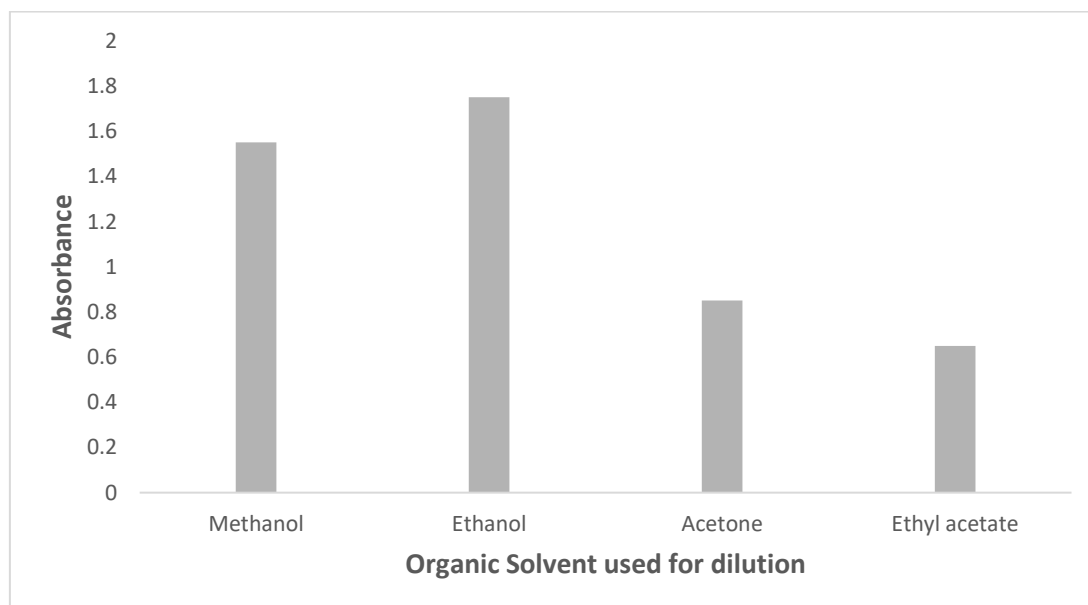


Figure 2: Effect of Organic Solvents on Complexation of Piroxicam with Fe(III).

3.2. Effect of pH

The effect of pH on the complexation of Piroxicam with Fe(III) and, ultimately, on the final absorbance at 505 nm was checked at acidic (pH=4), neutral (pH=7), and basic (pH=10) media. Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) that contains a carboxylic acid functional group (-COOH), which can act as a potential ligand for metal chelation. As shown in Figure 3, in the present work, the maximum absorbance was obtained at pH 4. This

was probably due to the fact that at low pH (acidic conditions), the carboxylic acid group in Piroxicam is more likely to be protonated, resulting in a negatively charged species. Fe(III) ions may have a higher affinity for the negatively charged ligand since the opposite charges can attract each other more strongly. Chelation can occur more readily at low pH due to the increased availability of negatively charged ligands.

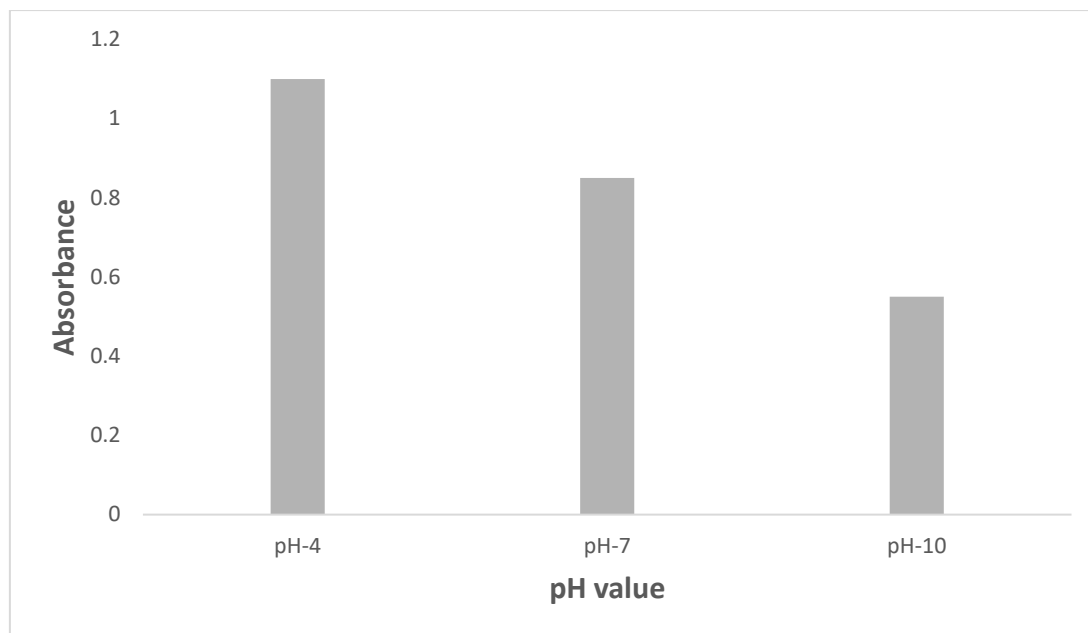


Figure 3: Effect of pH on Complexation of Piroxicam with Fe(III).

3.3. Effect of Concentration of Fe(III) Solution

The effect of the concentration of the iron(III) solution was checked by adding different volumes (1 to 10 mL) having concentrations 0.05 mM, 0.1 mM, 0.15 mM, 0.20 mM, 0.25 mM, 0.3 mM, 0.35 mM, 0.4 mM, 0.45 mM and 0.5 mM of ferric sulfate in a certain quantity of Piroxicam. As shown in Figure 4, the

absorbance reached a maximum when 4 mL of iron solution was added to 100 ppm of Piroxicam. Further addition of Fe(III) did not affect the final absorbance. Therefore, throughout this work, 5 mL of iron(III) solution was used to develop the red complex of Iron-Piroxicam.

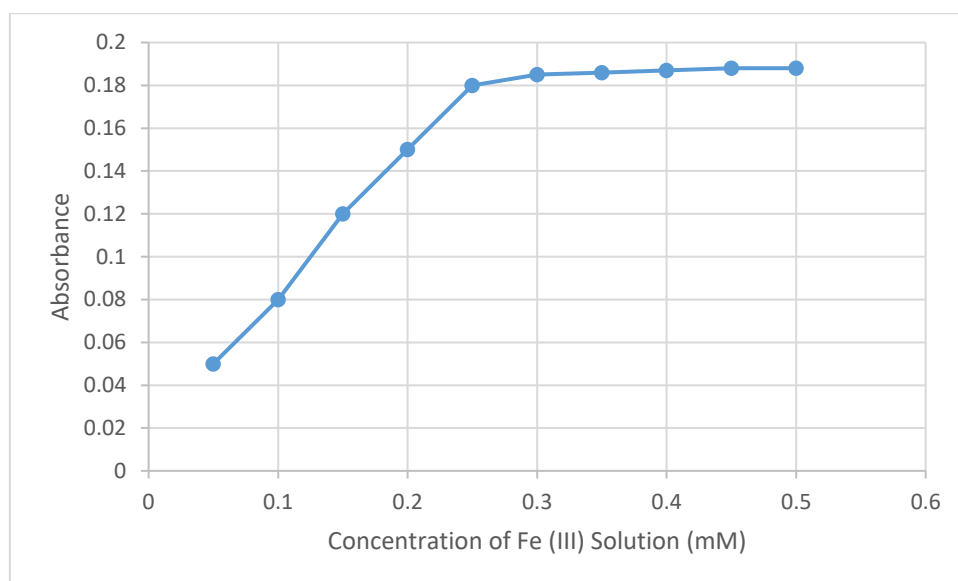


Figure 4: Effect of Concentration of Fe (III) Solution on Complexation.

3.4. Effect of Time and Temperature

The effect of temperature was checked by reacting the Piroxicam with iron(III) solution in the presence of pH-4 buffer and heating the contents at elevated temperature in a water bath before diluting with ethanol. It was observed that high temperature did not affect the final absorbance. Almost the same absorbance was monitored at 40 °C and 60 °C as was obtained at room temperature (25 °C). This reveals that the complexation between iron(III) and Piroxicam is completed at room temperature. The effect of time was checked by mixing the Piroxicam,

buffer, and Fe(III) solution in five different flasks and keeping them for various intervals of time before diluting with ethanol.

In all the flasks, the red color of the Piroxicam-iron (III) complex developed in the first five min, and then no change in absorbance was observed in the next hour. However, in this work, absorbance in all experiments was measured after 10 min of incubation with the reagents. The effect of time is shown in Figure 5.

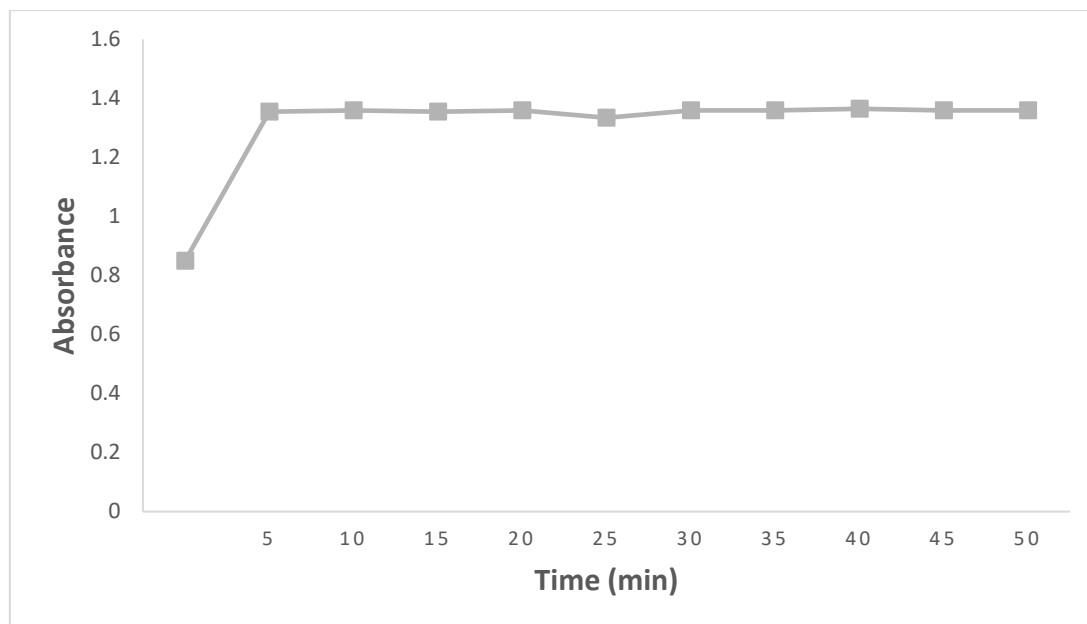


Figure 5: Effect of Time on Complexation of Piroxicam with Fe(III).

3.5. Effect of Order of Mixing the Reagents

This effect was checked by mixing Piroxicam, buffer, and Fe(III) solutions in different orders. Slightly better absorbance was observed when Piroxicam solution was taken first, and buffer was mixed with it. Lastly, an iron(III) solution was added to develop the red color of the complex. The slight enhancement

observed in absorbance in this order of mixing the reagents could be attributed to the fact that the addition of buffer may facilitate the protonation of Piroxicam prior to the addition of Fe(III) solution for complexation. The effect of the order of mixing the reagents is shown in Figure 6.



Figure 6: Effect of Order of Mixing the Reagents on Complexation.

3.6. Statistical Data

To validate the proposed method, ten samples of Piroxicam (50 ppm) were analyzed using the Image Scanning Densitometric method and the spectrophotometric method. The results obtained by both methods were used to calculate the analytical data, which are shown in Table 1. The analytical data show that in terms of accuracy and precision, the proposed method is almost comparable with the classical spectrophotometric method. In addition, the proposed method does not require a spectrophotometer and is thus free from instrumental errors.

3.7. Results of Commercial Formulations

The average results of Piroxicam content obtained by Image Scanning Densitometry and by Spectrophotometry with percentage errors are shown in Table 2. A close comparison has been found in the results obtained by both methods (Figure 7). Secondly, most of the results are close enough to the reported values given for the packing of various companies. Calibration curves with standard Piroxicam were plotted by both methods (Figures 8 and 9). The colored standard and sample solutions in the well plate are given in Figure 10.

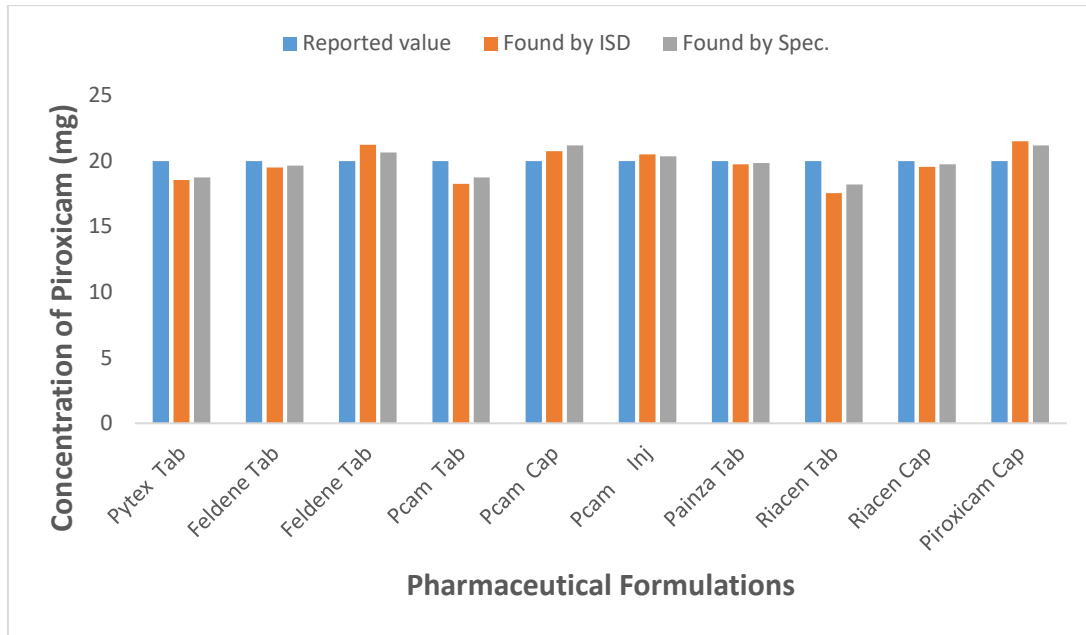


Figure 7: Piroxicam Quantities Reported and Found by ISD and Spectrophotometry.

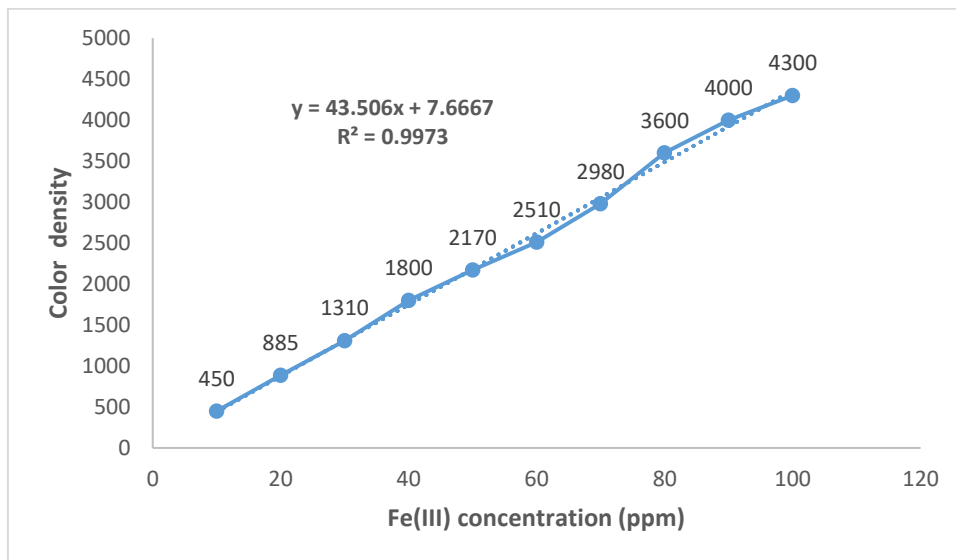


Figure 8: Calibration Curve for Piroxicam by image scanning densitometry.

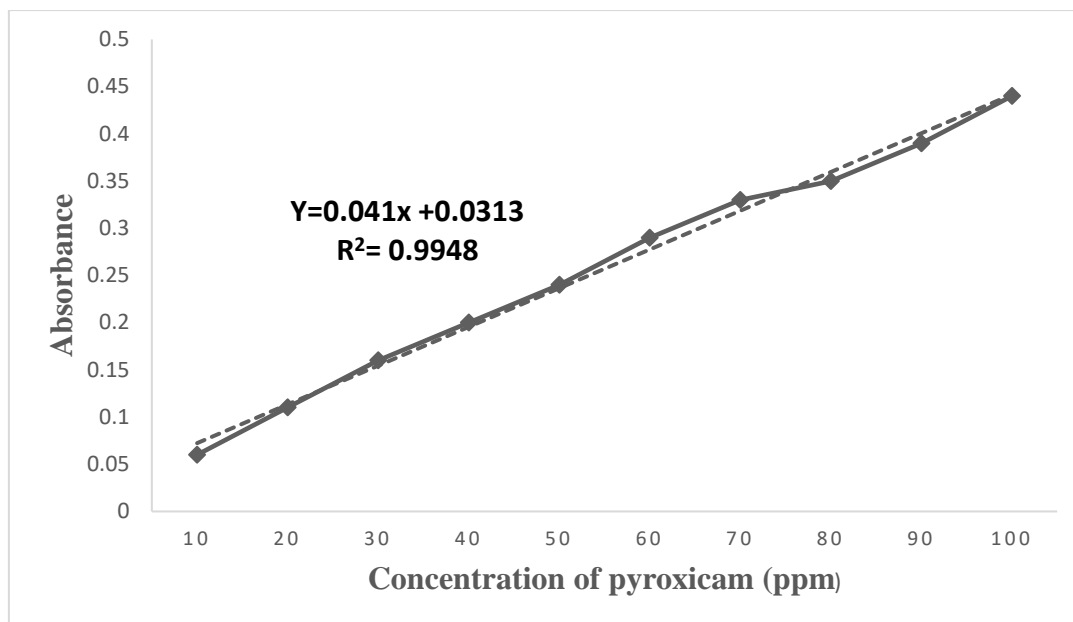


Figure 9: Calibration Curve for Piroxicam by Spectrophotometry.

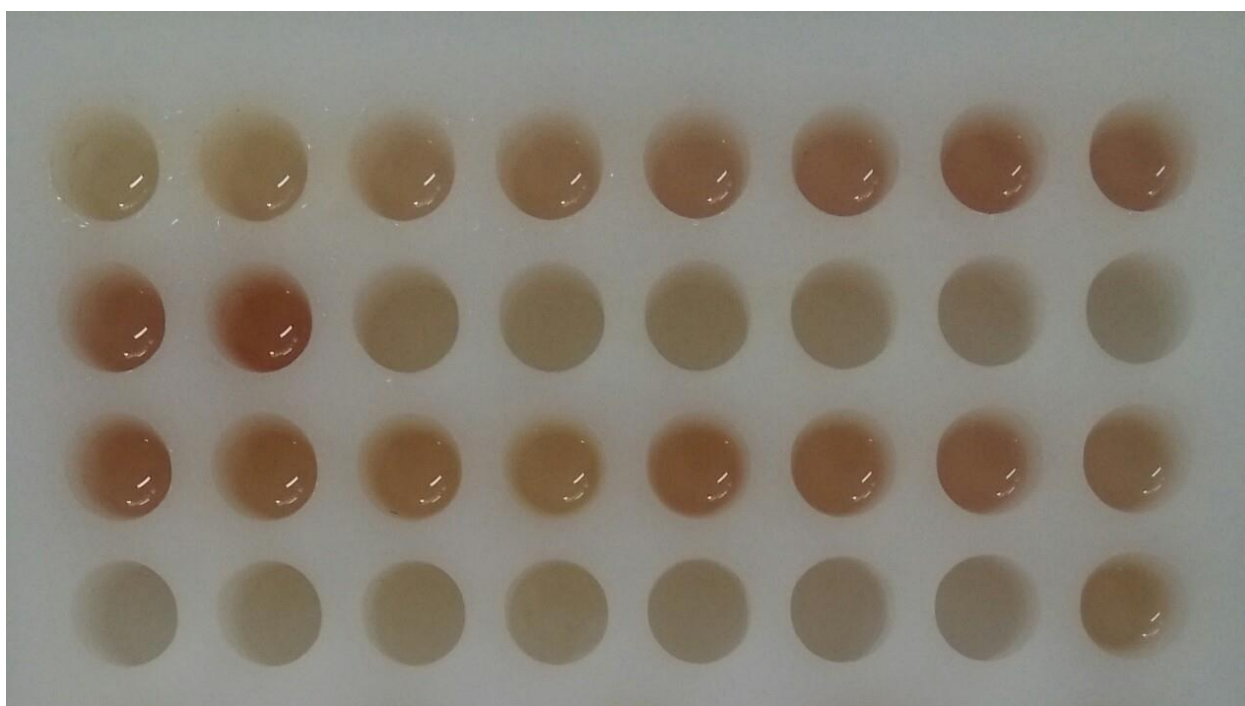


Figure 10: Standard and sample solutions in the Teflon well plate.

Interestingly, all the tablet formulations showed negative deviation from the reported value, whereas capsules and injection samples gave positive percentage error. This minute difference can be attributed to the presence of some fillers, which may

cause the extraction of the drug to not be 100% from the tablets. The analytical data given in Tables 1 and 2 showed the compatibility of the ISD method with the classical spectrophotometric method.

Table 1: Comparison of analytical data obtained by ISD and reference method.

No.	Parameter	Value Obtained by ISD method	Value Obtained by Ref. (Spec.) method
1.	Correlation Coefficient	0.9996	0.9952
2.	Standard Deviation	0.558	0.540
3.	Relative Standard Deviation	3.3644	3.3275
4.	Precision	3.28045	3.14582
5.	Accuracy	0.82684	0.80578

6.	LOD ($\mu\text{g/mL}$)	0.9570	0.9858
7.	LOQ ($\mu\text{g/mL}$)	2.9210	2.9578
8.	Confidence Level	1.865	1.758
9.	Multiple R	0.999468	0.995280
10.	R Square	0.9994	0.9948
11.	Adjusted R Square	0.996592	0.993680
12.	Standard Error	3.458210	3.584012
13.	Number of replicate samples	10	10

Table 2: Results of Commercial Formulations of Piroxicam by ISD and Reference Method.

No.	Commercial Formulation	Piroxicam Reported	Piroxicam found by ISD method (% error)	Piroxicam found by Spec. method (% error)
1.	Pytex Tablet	20 mg/tablet	18.55 (-7.25%)	18.75 (-6.25%)
2.	Feldene Tablet	20 mg/tablet	19.50 (-2.50%)	19.65 (-1.75%)
3.	Feldene Capsule # 323	20 mg/capsule	21.25 (+6.25%)	20.65 (+3.25%)
4.	Pcam Tablet	20 mg/tablet	18.25 (-8.75%)	18.75 (-6.25%)
5.	Pcam Capsule	20 mg/capsule	20.75 (+3.75%)	21.20 (+6.00%)
6.	Pcam Injection	20 mg/Injection	20.50 (+2.50%)	20.35 (+1.75%)
7.	Painza Tablet	20 mg/tablet	19.75 (-1.25%)	19.85 (-0.75%)
8.	Riacen Tablet	20 mg/tablet	17.55 (-12.25%)	18.20 (-9.00%)
9.	Riacen Capsule	20 mg/capsule	19.55 (-2.25%)	19.75 (-4.25%)
10.	Piroxicam Capsule	20 mg/capsule	21.50 (+7.50%)	21.20 (+6.00%)

4. CONCLUSION

Ten commercial formulations of various companies collected from the local market were analyzed for Piroxicam content by Image Scanning Densitometry (ISD) and by a reference spectrophotometric method. The statistical data and analytical results obtained by both methods, as shown in the Tables, revealed that ISD is a reliable and accurate method for the assay of drugs. In addition, this technique is fast, simple, cost-effective, and environment friendly and can be employed without any sophisticated spectrophotometer.

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