Research Article

วิธีโฟลอินเจคชั่นสเปคโทรฟลูออริเมตริกอย่างง่ายในการวิเคราะห์ไพร็อกซิแคม

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บทคัดย่อ

วิธีโฟลอินเจคชั่นสเปคโทรฟลูออริเมตริกอย่างง่ายในการวิเคราะห์ไพร็อกซิแคม

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วิธีโฟลอินเจคชั่นสเปคโทรฟลูออริเมตริกอย่างง่ายได้ถูกพัฒนาขึ้นสำหรับการวิเคราะห์ปริมาณไพร็อกซิแคม หลักการพื้นฐานของโฟลอินเจคชั่นเป็นการฉีดสารละลายมาตรฐานหรือตัวอย่างเข้าสู่ระบบการไหลแบบต่อเนื่องในกระแสของตัวพาซึ่งจะเคลื่อนที่นำสารไปสู่ตัวตรวจวัดคือสเปคโตรฟลูออริมิเตอร์โดยตรง **วิธีการศึกษา:** วิธีที่นำเสนอนี้ สารลายมาตรฐานหรือตัวอย่างปริมาตร 150 ไมโครลิตร จะถูกฉีดเข้าไปในกระแสของสารละลายตัวพา คือ กรดไนตริก 0.1 โมลต่อลิตร ด้วยอัตราเร็วการไหลที่เหมาะสม 4.0 มิลลิลิตรต่อนาที ปฏิกิริยาที่เกิดภายในท่อผสมสารละลายยาว 100 เซนติเมตร ถูกส่งผ่านไปสู่ตัวตรวจวัดสัญญาณแบบฟลูออริเมตริก ความเข้มของค่าฟลูออเรสเซ็นซ์ถูกบันทึกโดยใช้ค่าความยาวคลื่นของการกระตุ้นและการคายคลื่นแสงที่ 330 และ 440 นาโนเมตร ตามลำดับ **ผลการศึกษา:** กราฟมาตรฐานระหว่างความเข้มข้นไพร็อกซิแคม ในช่วง 0.1-4.0 ไมโครกรัมต่อมิลลิลิตร กับความเข้มของค่าฟลูออเรสเซ็นซ์ ความเป็นเส้นตรงภายในช่วงความเข้มข้นที่เหมาะสมมีสมการแสดงความสัมพันธ์ คือ $y = 92.946x + 0.2335$ และมีค่าสัมประสิทธิ์ของการวิเคราะห์ r^2= 0.9994 ค่าขีดจำกัดต่ำสุดของการตรวจวัด (LOD) มีค่าเท่ากับ 0.03 ไมโครกรัมต่อมิลลิลิตร ค่าเปอร์เซ็นต์การคำนวณย้อนกลับมีค่าในช่วง 98.65%-100.48% เปอร์เซ็นต์ และความสามารถในการวัดซ้ำมีค่าส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า 0.43 เปอร์เซ็นต์ การศึกษาผลของสารปรุงแต่งในทางเภสัชภัณฑ์ พบว่าไม่มีผลใดๆต่อการวิเคราะห์ปริมาณไพร็อกซิแคม ผลของการวิเคราะห์ตัวอย่างไพร็อกซิแคมด้วยวิธีที่นำเสนอเมื่อเปรียบเทียบกับวิธีอ้างอิงแล้วพบว่าไม่มีความแตกต่างกันในเชิงสถิติด้วยความเชื่อมั่นที่ 95 เปอร์เซ็นต์ (n=10) **สรุปผล:** วิธีที่นำเสนอนี้ ให้ความรวดเร็ว ประหยัด มีอัตราเร็วในการวิเคราะห์ 90 ตัวอย่างต่อชั่วโมง และสามารถที่จะนำไปใช้ประโยชน์ในการวิเคราะห์ไพร็อกซิแคมในรูปแบบแคปซูลได้

คำสำคัญ: โฟลอินเจคชั่น, การตรวจวัดแบบฟลูออริเมตริก, ไพร็อกซิแคม

Simple Flow Injection Spectrofluorimetric in Determination of Piroxicam

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Abstract

Simple Flow Injection Spectrofluorimetric in Determination of Piroxicam

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A simple flow injection spectrofluorimetric method has been developed for the determination of piroxicam. The principle is based on the injection of a standard or sample solution into a moving, nonsegmented continuous carrier stream, which is then transported toward a spectrofluorometer detector. **Method:** The presented method was developed on the injection of 150 µl standard or sample solution of piroxicam into the carrier stream of 0.1 mol l^{-1} nitric acid with the optimum flow rate at 4.0 ml min^{-1}. The reaction is occurred in 100 cm of mixing coil length which was passed through to the fluorimetric detection unit. The fluorescence intensity was monitored using excitation and emission wavelength at 330 and 440 nm, respectively. **Results:** The calibration curve was established in concentration ranges 0.1-4.0 µg ml^{-1} of piroxicam which was plotted against fluorescence intensity. For linearity within the optimum range, regression equation was shown y = 92.946x + 0.2335, and the coefficient of determination (r^2) was 0.9994. The limit of detection (LOD) was 0.03 µg ml^{-1}. The percentage recoveries of proposed method had shown in range of 98.65%-100.48% and repeatability was found the relative standard deviation lesser than 0.43%. The effect of excipients which are usually added to pharmaceutical formulations was investigated on piroxicam analysis and shown none effected for determination of piroxicam. The difference between the results of proposed and reference methods was figured out statistically and showed no statistical difference at a 95% confidence level (n=10). **Conclusion:** The proposed method is fast and reasonably economic with the rate of 90 sample h^{-1}, and could be useful for routine analysis of piroxicam in capasules.

Keywords: Flow injection, Fluorimetric detection, Piroxicam

Introduction

Piroxicam; C15H13N3O4S, [4-hydroxy-2-methyl-3-(pyrid-2-yl-carbamoyl)-2H-1,2-benzothia zine 1,1-dioxide] is a traditional of non-steroidal anti-inflammatory drugs (NSAIDs). It is odorless, slightly yellow and crystalline. Piroxicam have long half-life and effective response in the treatment of many disease such as rheumatoid arthritis, gout, osteoarthritis and musculoskeletal disorders (Brunton LL, *et al.*, 2017). Although, this drug is vastly employed in human and veterinary medicine, unfortunately, some common side effects including headache, gastrointestinal disorder, dizziness, palpitations, skin rashes, and tinnitus have been observed in the patients consuming this drug (Paulus HE, *et al.*, 1987). This drug has been stated to also exhibit chemo suppressive and chemo protective properties, which supported their inclusion in the treatment of some cancer types such as colorectal cancer. Hence NSAIDs relevance in therapeutics, the assessment of drugs such as piroxicam in pharmaceutical forms is a mandatory step for quality control analysis (Bahmani MM, *et al.*, 2022).

Several analytical methods have been reported in the scientific literature for determination of piroxicam in pharmaceutical preparations including UV-visible spectrophotometry (Kaur M, *et al.*, 2022), spectrofluorimetry (Damiani PC *et al.*, 1998), voltammetry (Santos AM, *et al.*, 2019), biosensor (Kalambate PK, *et al.*, 2021), liquid chromatography (El-Hay SSA, *et al.*, 2022) liquid chromatography-mass spectrometry (Mashal MS, *et al.*, 2022) and flow injection with UV-visible detector (Abed RI and Hadi H, 2020). However, a report of flow injection method in couple with spectrofluorometric detection for the determination of piroxicam has not been yet available in the literature.

The principle of Flow Injection Analysis (FIA) assembly is allow the reproducible insertion of a volume of sample into a carrier or carrier-reagent stream without altering its flow-rate. Also, the sample volume should be modifiable at will and the carrier stream is flow consistently in a pulse-free manner through a conduit of uniform cross-section. The manifold into which the sample is inserted should be flexible enough to allow implementation of a wide variety of chemical reaction or tests involving merging of the sample stream with one or more reagent streams. The transient signal produced by the detector should be not only instantaneous and reproducible, but also high sensitive (Calatayud JM, 1996).

In the present work, a new flow injection spectrofluorimetric method is proposed for the determination of piroxicam. The method is based on the injection standard or sample solution of piroxicam into the carrier stream of 0.1 mol l^{-1} nitric acid. The fluorescence intensity was recorded using excitation and emission wavelength at 330 and 440 nm, respectively. This method could be used as the alternative method for determination piroxicam. The procedure is simple no need sophisticate instrumentation and not time consuming. The proposed method was successfully for determination of piroxicam in capsules.

Materials and Methods

All chemicals were of analytical reagent grade and were used without further purifcations. The piroxicam standard and nitric acid were purchased from Sigma-Aldrich; USA and RCI Labscan; Thailand, respectively. Others mineral acid (perchloric, phosphoric, sulfuric and hydrochloric acid) were provided from Ajax Finechem; Australia. All selective excipients (magnesium stearate, lactose, aerosol and starch) were purchased from Merck; Germany. Piroxicam standard solution was prepared with 0.1 mol l^{-1} nitric acid. To prepare 1000 ml of mol l^{-1} nitric acid, you will need to dilute 6.97 ml of 65% nitric acid with distillation water to make a total volume of 1000 ml with graduate volumetric flask.

The flow injection (FI) manifold (Figure 1) consisted of a peristaltic pump (Eyela® MP3A, Tokyo Rikakikai Co. Ltd., Japan), the standard or sample solution was injected into the FI system via a four way PTFE rotary valve with a 150 µl sample loop (Rheodyne® model 5041, Cotati, CA). PVC tubing (Elkay®, Galway, Ireland) with 0.8 mm i.d. was used as a flow line for carrier solution which was used for

merging standard or sample and carrier streams. A mixing coil used was made from PTFE tubing, 0.8 mm i.d. and 100 cm in length for the recommended configuration. The FI peaks were acquired by using a fluorescence detector (Thermo Separation Product®, TSP FL-2000, USA), coupled with a chart recorder (Kipp & Zonen® BD50, The Netherlands).

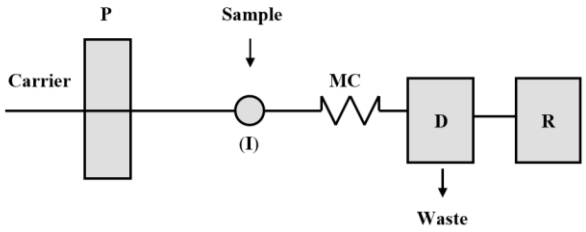

Figure 1 Flow injection-spectrofluorometry (FI-SF) manifold for piroxicam determination in pharmaceutical preparation; (P) peristaltic pump, (I) injection port, (MC) mixing coil, (D) fluorescence detector and (R) recorder

Procedures

Standard solution and sample preparation

A stock piroxicam standard solution of 100 µg ml^{-1} was prepared by accurately weighing 0.0100 g of piroxicam standard in 0.1 mol l^{-1} nitric acid and the volume was adjusted to 100 ml of graduate mark to provide a final concentration of 100 µg ml^{-1}. The stock standard solution was prepared once a day and stored in the refrigerator before used.

The sample of piroxicam capsules (10 mg/capsule) were purchased from drug stores in Khon Kaen Province, Thailand. The powder from 20 capsules of piroxicam was collected and an amount equivalent to 10 mg of piroxicam was accurately weighed using an analytical balance. The drug sample was dissolved in 100 ml of 0.1 mol l^{-1} nitric acid and filtered through Whatman No 41 filter paper afterward the sample solution was diluted in 0.1 mol l^{-1} nitric acid to obtain the appropriate concentrations (1 µg ml^{-1} of piroxicam) for analysis.

Recommended procedure

Using the experimental setup as shown in Figure 1, a 150 µl standard or sample solution containing piroxicam was injected into a stream of 0.1 mol l^{-1} nitric acid solution at the optimum flow rate of 4.0 ml min^{-1}. Subsequently, the sample zone flowed through the 100 cm in length of mixing coil, where the standard or sample was mixed with carrier. The fluorescence intensity was monitored using excitation and emission wavelength at 330 and 440 nm, respectively, the FI signal was recorded using a chart recorder.

Results and Discussion

The proposed flow system was undertaken development of FI procedure for piroxicam determination based on the fluorescence intensity was emitted in an acid solution. The experimental parameters dealing with the FI method development were optimized by an univariate method and follow by using ICH guideline Q2(R1), (ICH guideline, 2005). The variable by variable method was applied to select the optimum conditions for the flow injection-spectrofluorimetic (FI-SF) determination of piroxicam.

Effect of fluorescece spectra

The fluorescece spectra of piroxicam was studied. It was found that a suitable solution for dissolving this drug was 0.1 mol l^{-1} of nitric acid. The solution of piroxicam was took for studying the fluorescene spectrum which was scanned over the range of 200–600 nm using a spectrofluorimeter. In order to achieve the greatest sensitivity, measurements were used excitation and emission wavelength at 330 and 440 nm, respectively.

Effect of acid types and concentration

The effect of 0.1 mol l^{-1} nitric (HNO_3), hydrochloric (HCl), perchloric ($HClO_4$), sulfuric (H_2SO_4) and phosphoric acid (H_3PO_4) solution were studied. The relative fluorescence intensities were 100.0, 73.4, 41.9, 28.2 and 26.7%, respectively. The presence of HNO_3 gave the highest fluorescence intensity, so was chosen for subsequent studies (Figure 2).

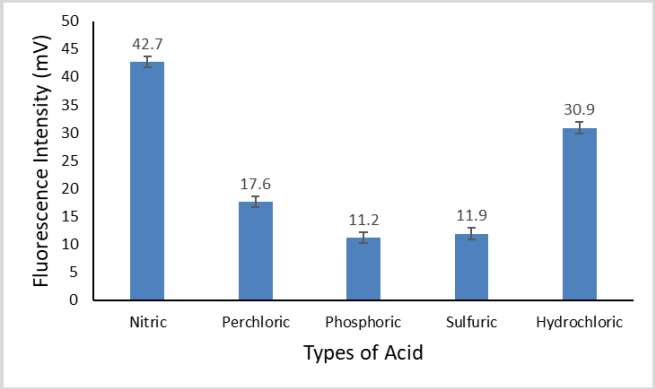

Figure 2 Effect types of acid on the mean of fluorescence intensity (n=5) of 0.6 µg ml^{-1} piroxicam standard solution.

The concentration of HNO_3 solutions was optimized. Various concentrations over the range 0.05 to 1.0 mol l^{-1} were investigated, and the nitric concentration chosen for further studies was 0.1 mol l^{-1} because this concentration gave a maximum fluorescence intensity and can use as FI-carrier in this present method completely (Figure 3).

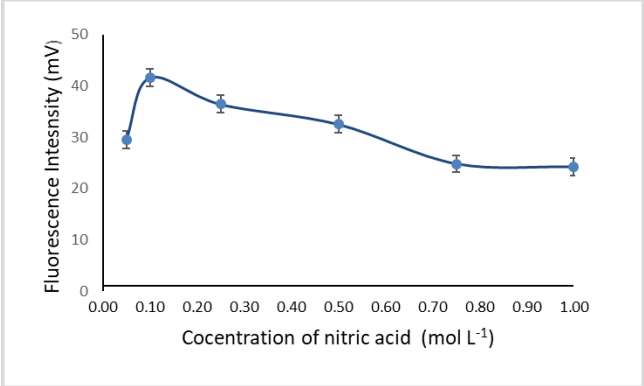

Figure 3 Effect concentration of nitric acid on the mean of fluorescence intensity (n=5) of 0.6 µg ml^{-1} piroxicam standard solution.

Effect of mixing coil length and injection volume

The mixing coil tubing length should be long enough to permit effective mixing of the standard or sample in which the carrier to be favored. This study was carried out at various mixing coil tubing lengths between 25 and 250 cm, injection loop volumes between 25 and 250 µl on the fluorescence intensities were investigated. It was found that the fluorescence intensity increased with the mixing coil length up to 100 cm, and the mixing coil lengths of 25, 50, 100, 150, 200 and 250 cm provided the fluorescence intensity of 45.0, 67.2, 73.2, 57.4, 50.6 and 49.6 mV, respectively (Figure 4).

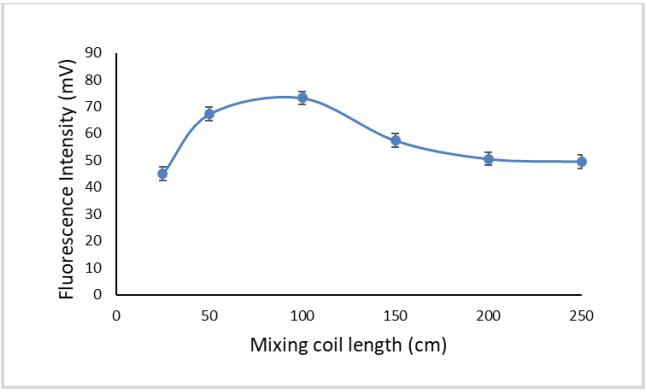

Figure 4 Effect of mixing coil length on the mean of fluorescence intensity (n=5) of 0.6 µg ml^{-1} piroxicam standard solution.

The sample injection volume must be large enough to contain sufficient amounts of the analyte to move in the carrier stream leading to the suitable response in the calibration range. The influence of the sample or standard volume on the fluorescence intensity was investigated by injecting the standard solution with varying volumes in the range 25–250 µl of 0.6 µg ml^{-1} piroxicam into the FI system. The results shown that fluorescence intensity increased from 30.0 to 82.2 mV when increasing the injection volume from 25 to 250 µl. It was found that the fluorescence intensity increased with the injection volume up to 150 µl, and the injection volume of 25, 50, 100, 150, 200 and 250 µl produced the fluorescence intensity of 30.0, 36.1, 47.5, 79.2, 81.4 and 82.2 mV, respectively. The appropriate fluorescence intensity was reached at 150 µl. The most suitable mixing coil length and injection loop volume values for further use were 100 cm and 150 µl, respectively (Figure 5). It was found that the solution of piroxicam in 0.1 mol l^{-1} nitric acid can be exhibited the high fluorescence and reduced the dispersion effect on flow system.

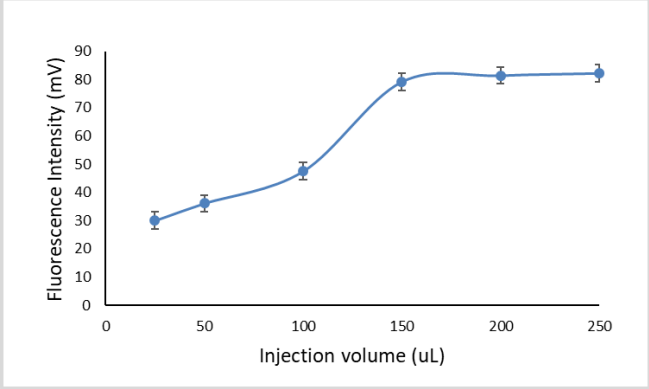

Figure 5 Effect of injection volume on the mean of fluorescence intensity (*n*=5) of 0.6 µg ml^{-1} piroxicam standard solution.

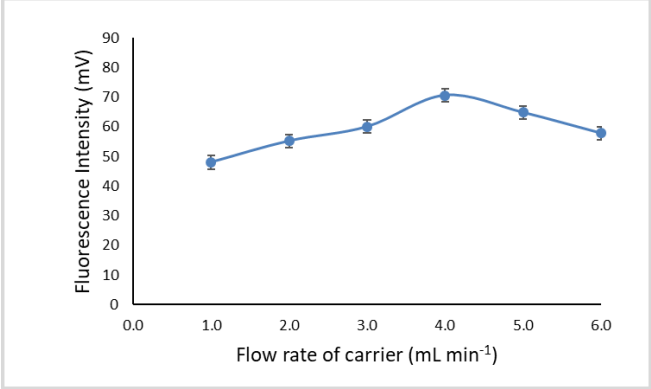

Figure 6 Effect of carrier flow rate on the mean of fluorescence intensity (*n*=5) of 0.6 µg ml^{-1} piroxicam standard solution.

Effect of carrier flow rate

The effect of flow rate of carrier solution (0.1 mol l^{-1} nitric acid) and standard solution (0.6 µg ml^{-1}) was investigated over the range 1.0-6.0 ml min^{-1} of carrier stream. It was found that the fluorescence intensity increased with the carrier flow rate up to 4.0 ml min^{-1}, and the carrier flow rate of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml min^{-1} were giving the fluorescence intensity of 48.0, 55.2, 60.0, 70.6, 64.8 and 57.8 mV, respectively. Thus, 4.0 ml min^{-1} carrier solution was regarded as the optimum flow rates (Figure 6).

Analytical Characteristics

Calibration curve

Under the optimum conditions (Table 1), linear calibration graphs were obtained for 0.1-4.0 µg ml^{-1} of piroxicam. Over the above concentration range, linear regression analysis of fluorescence intensity value (y) versus drug concentration (x) yield equations y = 92.946x + 0.2335. The coefficient of analysis of two variables was 0.9994 (*n*=5), (Figure 7).

The detection limit was defined as the concentration of analyte that gave the signal that was different from the blank by an amount equal to three times the standard deviation of the blank signal (3σ). It was found to be 0.03 µg ml^{-1} piroxicam. The quantification limit is defined as the analyte producing a signal that is at least 10 times the standard deviation of the blank signal (10σ), and was found to be 0.10 µg ml^{-1} piroxicam.

Table 1 Optimum conditions for piroxicam determination

Conditions	Studied range	Optimum conditions
FI-SF wavelength (nm)	200-600	λ_{ex} =330 , λ_{em}=440
Types of acid	HCl, HNO_3, $HClO_4$, H_2SO_4, H_3PO_4	HNO_3
Nitric acid concentration (mol l^{-1})	0.05, 0.10, 0.25, 0.50, 0.75, 1.00	0.10
Mixing coil (cm)	25, 50, 100, 150, 200, 250	100
Injection volume (µl)	25, 50, 100, 150, 200, 250	150
Carrier flow rate (ml min^{-1})	1.0-6.0	4.0

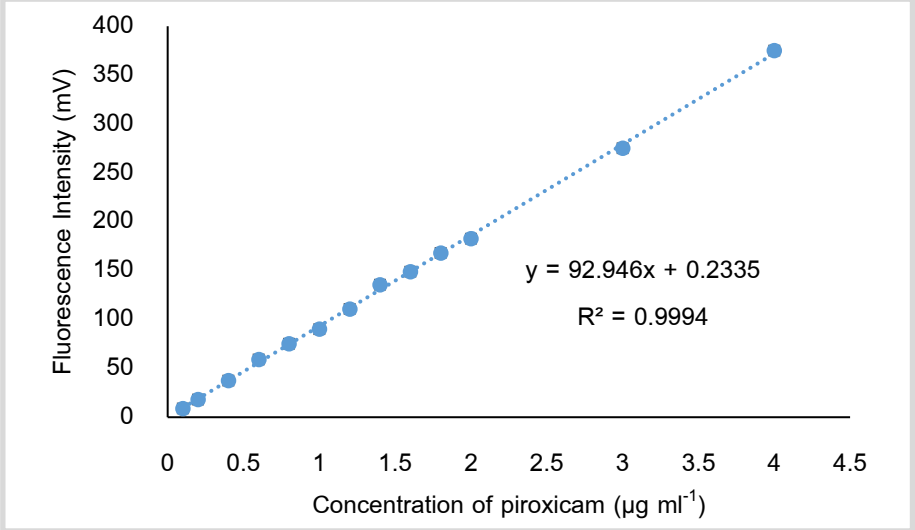

Figure 7 Standard calibration of piroxicam in the range of 0.1-4.0 µg ml^{-1}.

Precision and accuracy

The relative standard deviation of the proposed method from 12 repeated of piroxicam 0.2, 0.8 and 2.0 µg ml^{-1} were carried out using standard addition method. The percentage of relative standard deviation of intra-day was 0.25, 0.19 and 0.32 (*n*=12) and inter-day was 0.37, 0.31 and 0.42 (*n*=12), respectively. The recoveries percentage of accuracy for the proposed method was studied on 0.2, 0.8 and 2.0 µg ml^{-1}. The results were 98.65 %, 99.21% and 100.48% (*n*=5) for intra-day and 98.74%, 99.58% and 100.28% (*n*=5) for inter-day, respectively. The analytical characteristics of this proposed method was summarized in Table (2). The percentage recoveries of proposed method had shown in range of 98.65%-100.48% and repeatability was found the relative standard deviation lesser than 0.43% which were proved that this present method was provide accurate and precise results.

Table 2. Analytical characteristics for piroxicam analysis using FI-SF method

Parameters	Optimum value	
Linearity of calibration curve, µg ml^{-1}	0.1 – 4.0	
Linear regression equation (*n*=5)	$y = 92.946x + 0.2335$	
Correlation coefficient, r^2	0.9994	
Limit of detection (LOD), µg ml^{-1}	0.03	
Limit of quantification (LOQ), µg ml^{-1}	0.10	
Repeatability (*n*=12); %RSD, µg ml^{-1}	Intra-day	Inter-day
0.2	0.25 %	0.37 %
0.8	0.19 %	0.31 %
2.0	0.32 %	0.43 %
Percentage recoveries (*n*=5), µg ml^{-1}		
0.2	98.65 %	98.74 %
0.8	99.21 %	99.58 %
2.0	100.48 %	100.28 %

Selected excipients studies

Some excipients are usually added in pharmaceutical formulations. There was an investigation of interference of excipients on pioxicam analysis by the presented method. The selected excipients were magnesium stearate, lactose, aerosol and starch. Piroxicam (0.6 µg ml^{-1}) was prepared in 0.1 mol l^{-1} nitric acid, in which the concentrations of selected excipients were added 5, 10 and 50 times greater than the concentration of piroxicam standard. These were found that the relative percentage of fluorescence intensity were in range of 97.1%-99.2%. The obtained data was showed a slightly effect from these excipients for the determination of piroxicam (Table 3)

Table 3. Effects of excipients on piroxicam 0.6 µg ml^{-1}.

Types of excipients	Amount of excipients	Fluorescence intensity (*n*=5)	Relative Percentage (%)
Piroxicam with none excipients		60.76	100.0
Magnesium stearate	5 times	60.08	98.9 ± 0.54
	10 times	59.39	97.7 ± 0.83
	50 times	59.14	97.3 ± 1.09
Lactose	5 times	60.08	98.9 ± 0.54
	10 times	59.70	98.3 ± 1.03
	50 times	59.70	98.3 ± 1.34
Aerosil	5 times	59.95	98.7 ± 1.03
	10 times	59.89	98.6 ± 1.30
	50 times	59.76	98.4 ± 1.54
Starch	5 times	60.26	99.2 ± 0.75
	10 times	59.39	97.7 ± 0.89
	50 times	58.97	97.1 ± 1.14

Application for commercial samples

The proposed method was developed for the examination of 10 commercial products which contain 10 mg of piroxicam in each capsule. The results of the proposed method were estimated the similarity with that batch spectrofluorometric method (reference method). Piroxicam was determined the fluorescence intensity using excitation and emission wavelength at 330 and 440 nm, respectively, for reference method (Damiani PC *et al.*, 1998). The accuracy of proposed method was calculated statistically by using the Student's t-test at a 95% confidence level. The values are expressed in Table 4. The t-calculation and t-critical value are 1.68 and 2.26, respectively. It was found that the results obtained by both methods showed no statistical difference of those methods at the 95% confidence level (Miller JN and Miller JC, 2010). The advantages of this proposed method may be summed up as follows: (1) Its simplicity compare to the classical liquid chromatographic method. (2) This method uses simple instrumentation. (3) This technique may be applying for some scientific units where do not have UV-Visible spectrophotometer and liquid chromatographic unit in laboratory.

Table 4. Accuracy of proposed method compared with reference method for determination of piroxicam

Commercial samples	Piroxicam (10 mg/capsule); mean ± sd.	
	Proposed FI-SF method	Reference method
1	9.92 ± 0.05	9.75 ± 0.03
2	10.06 ± 0.05	9.77 ± 0.05
3	9.92 ± 0.05	9.76 ± 0.09
4	10.19 ± 0.11	10.21 ± 0.07
5	10.28 ± 0.05	10.23 ± 0.06
6	10.18 ± 0.08	10.19 ± 0.16
7	10.06 ± 0.05	9.76 ± 0.09
8	10.18 ± 0.08	10.22 ± 0.11
9	10.08 ± 0.08	10.14 ± 0.06
10	10.13 ± 0.13	10.20 ± 0.07
t-Test at 95% confidence level		
t–calculation		1.68
t-critical value (*n-1)*		2.26

Conclusion

The proposed FI spectrofluorometric method has proved to be simple and cost-effective for determination piroxicam in pharmaceutical formulation. This FI method is based on the fluororescence intensity was recorred in which a small volume of piroxicam was injected into a carrier stream of 0.1 mol l^{-1} nitric acid and fluorescence intensity monitored using excitation and emission wavelength at 330 and 440 nm, respectively. The linearity of the calibration graph is in the useful range for quantitation of piroxicam, with detection limit of 0.03 µg ml^{-1} which were better than the previously published work including spectrophotometry (Pascual-Reguera *et al.*, 2002; Sversut RA *et al.*, 2020), and fluorescence (Zhang J. *et al.*, 2023). This method is fast and reasonably economic, providing a good sample frequency of 90 h^{-1}, and could be useful for routine analysis of piroxicam in capsules.

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