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Flow-injection spectrophotometric determination of piroxicam

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Abstract: Two flow-injection analysis (FIA) methods are proposed for the determination of piroxicam. The first involves measurement of the UV absorbance of a solution containing the drug, methanol and hydrochloric acid at 332 nm; in the second method a Fe(III)–piroxicam complex is formed in a methanolic medium and the absorbance is measured at 520 nm. In both methods, the peak height is used as a quantitative parameter and piroxicam is determined over the ranges 0.5–15 and 30–500 μg ml^{-1}, respectively. The methods have been applied to the routine determination of the drug in dosage forms.

Keywords: *Piroxicam; FIA; spectrophotometry; pharmaceuticals.*

Introduction

Piroxicam, 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (PX), is a non-steroidal anti-inflammatory drug belonging to a new class of compounds called oxicams [1]. It is widely used in the treatment of patients with rheumatological disorders [2].

Several analytical procedures have been described for the analysis of piroxicam, especially spectrophotometric [3–9] and chromatographic methods [10–16]. However, no studies have been reported on the determination of piroxicam using flow-injection techniques.

Flow-injection analysis (FIA) is characterized by its simplicity, speed and the use of inexpensive equipment. Its results are accurate and precise. However, in most of the FIA methods reported the preliminary sample preparation steps are not automated. FIA is a major alternative to manual analytical methods, with clear advantages in terms of the short time required for each assay.

The aim of this study was the development of two simple, inexpensive and rapid FIA methods for use in the routine determination of piroxicam in pharmaceuticals. The proposed procedures are based on the ultraviolet absorption spectrum of piroxicam and on the visible absorption of the complex formed between piroxicam and Fe(III).

Experimental

Apparatus

The FIA system comprised a Gilson HP4 peristaltic pump with isoversinic flow tubes of 2 mm i.d. (Worthington, OH, USA), an Omnifit injection valve (NY, USA), a Hellma 18 μl flow cell (Jamaica, NY, USA) and a Pye–Unicam spectrophotometer (Cambridge, UK) as the detector. Poly(tetrafluoroethylene) (PTFE) connecting tubing of 0.5 mm i.d. and various end-fittings and connectors (Omnifit) were used.

Reagents

All chemicals were of analytical reagent grade and the solutions were prepared with double-distilled water.

Piroxicam stock solution (0.5 mg ml^{-1}). This solution was prepared by dissolving 50 mg of piroxicam (Sigma, St Louis, MO, USA) in 100 ml of methanol (anhydrous) (Merck). Working standard solutions were prepared by suitable dilution of the stock solution with methanol.

Iron(III) chloride solution (5×10^{-3} M). This reagent was prepared by dissolving 0.3378 g of $FeCl_3.6H_2O$ (Merck) in methanol to 250 ml.

Hydrochloric acid (0.1 M). This was pre-

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pared by dilution of 5 ml of concentrated HCl (Merck) in methanol to 500 ml.

Dosage forms of piroxicam. (1) Doblexan capsules (Organon Lab., Spain): piroxicam 10 mg with lactose and other excipients; (2) Improntal capsules (Fides Lab., Spain): piroxicam 20 mg with excipients; (3) Sasulen capsules (Andreu Lab., Spain): piroxicam 20 mg with excipients; (4) Feldene tablets (Pfizer Lab.): piroxicam 20 mg with lactose and other excipients; (5) Feldene ampoules (Pfizer Lab.): piroxicam 20 mg with excipients in 2 ml of solution; (6) Improntal cream (Fides Lab.): piroxicam 0.5 g with excipients in 100 g; (7) Feldene suppositories (Pfizer Lab.): piroxicam 20 mg with excipients.

Recommended procedures for calibration

The flow-injection system is shown in Fig. 1. Samples were pumped into a sample loop of 200 μl for the UV–FIA method or 160 μl for the vis–FIA method, and then injected into an inert carrier stream of methanol. The solutions of 0.1 M HCl in methanol or 5×10^{-3} M Fe(III) in methanol were mixed with the carrier stream at the down-stream confluence point. The absorbances were measured at 332 nm in the first method or 520 nm in the second. A calibration graph was prepared by plotting the peak height (h) versus piroxicam concentration.

Procedure for the assay of dosage forms

Tablets, capsules, cream and ampoules. The contents of at least 10 capsules or 10 finely ground tablets were weighed and mixed. An amount of the tablet powder, capsule powder, ampoule solution or cream equivalent to 10 mg of piroxicam was weighed or measured accurately, dissolved in methanol and any remaining residue was removed by filtration. The clear solution was diluted to 50 ml with methanol in a calibrated flask and analysed by the visible FIA procedure. For the UV–FIA method, 5 ml of the solution was diluted to 100 ml with methanol and the described procedure was applied.

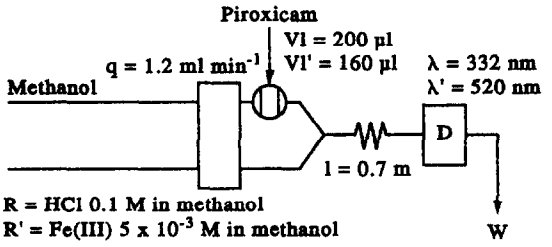

Figure 1
FIA manifolds for the determination of piroxicam.

Suppositories. At least 10 suppositories were weighed, cut into small pieces and transferred to a small porcelain dish. They were melted by stirring in a water bath until homogeneous and cooled; then weighed portions equivalent to 10 mg piroxicam were transferred into a beaker, melted and dissolved in methanol by stirring using a magnetic stirrer at 60°C for 5 min. The solution was cooled, filtered, diluted to 50 ml with methanol in a calibrated flask and analysed by the visible FIA procedure. For the UV–FIA procedure, 5 ml of the solution was diluted to 100 ml before applying the recommended procedure.

Results and Discussion

The UV absorption spectra of piroxicam in methanol (curve 1) and in methanolic hydrochloric acid (curve 2) are shown in Fig. 2. They show that piroxicam has a well defined absorption maximum in both media at 332 nm. Because a higher molar absorptivity (3.06×10^4 l mol^{-1} cm^{-1}) was shown in methanolic hydrochloric acid this medium was selected for subsequent studies.

Figure 3 shows the absorption spectra of piroxicam in methanol (curve 1), Fe(III) in methanol (curve 2) and piroxicam in the presence of Fe(III) in methanol (curve 3). In the third solution a new absorption maximum appeared at 520 nm, which was due to the formation of a Fe(III)–piroxicam complex.

The influence of the acidity on the formation of this complex was studied. Higher absorbance values were obtained when acidity decreased. The maximum absorbance was found in a methanolic medium, which was selected for subsequent studies. The molar ratio method was used to ensure that the stoichiometry of the Fe(III)–piroxicam complex in methanol was 1:1 with a molar absorptivity of 575 l mol^{-1} cm^{-1}.

Measurements of the absorbance of piroxicam at 332 nm and the absorbance of the Fe(III)–piroxicam complex at 520 nm were used to develop two spectrophotometric FIA methods for determining the drug.

The design of the manifolds shown in Fig. 1

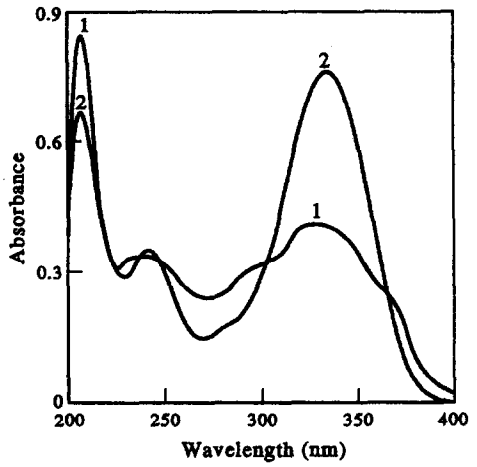

Figure 2
UV absorption spectra of (1) 8.3 μg ml^{-1} piroxicam in methanol and (2) 8.3 μg ml^{-1} piroxicam in 3.3×10^{-2} M methanolic HCl.

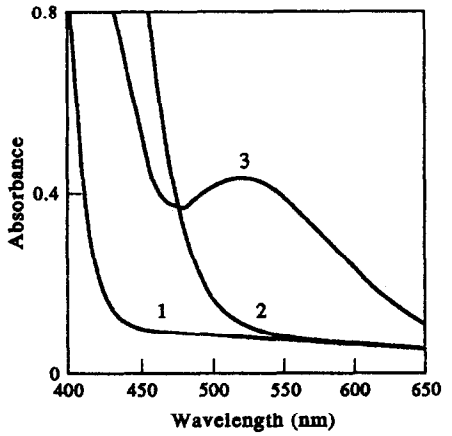

Figure 3
Absorption spectra of (1) 250 μg ml^{-1} piroxicam in methanol; (2) 2.5×10^{-3} M Fe(III) in methanol; (3) 250 μg ml^{-1} (7.5×10^{-4} M) piroxicam and 2.5×10^{-3} M Fe(III) in methanol.

is simple. The sample is injected into a methanol stream, which is then mixed with a stream of 1 M HCl in methanol and the absorbance is measured at 332 nm in the case of the UV–FIA method. For the vis–FIA method a stream of Fe(III) dissolved in methanol is used and the absorbance is measured at 520 nm. In the absence of the drug (blank) no signal is obtained. In both methods, the presence of the piroxicam causes an increase in the analytical signal, which is proportional to its concentration.

The use of FIA as an alternative to existing methods for the determination of piroxicam is dependent on optimization of this system to achieve maximum peak height, with low residence time and minimum dispersion. As a consequence, several experiments were conducted in order to establish the optimum conditions to operate the FIA manifold.

Figures 4 and 5 show the effects of the loop size, reactor length and flow rate on the peak height for piroxicam alone and for the Fe(III)–piroxicam complex, respectively. An increase in loop size produces an increase in peak height to reach a maximum and constant value in both cases [Figs 4(A) and 5(A)]. A loop size of 200 or 160 μl was chosen; at these volumes peak-height maxima are obtained with no excessive waste of sample.

The influence of reactor length was studied from the minimum distance possible between injection valve and detector up to 5 m. The

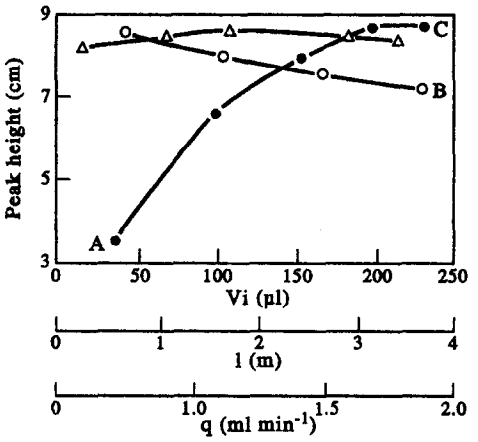

Figure 4
Effect of the loop size (A), reactor length (B) and pumping rate (C), on the peak height in the UV–FIA method. Sample injected: 5 μg ml^{-1} piroxicam.

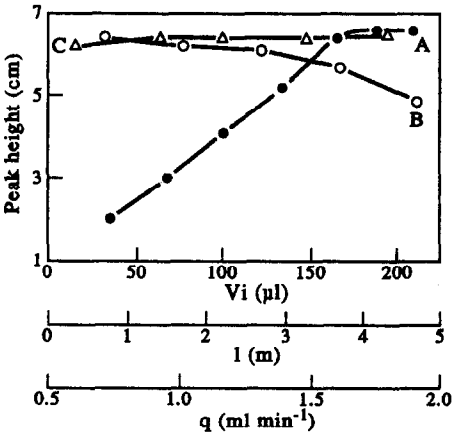

Figure 5
Effect of loop size (A), reactor length (B) and pumping rate (C), on the peak height in the vis–FIA method. Sample injected: 200 μg ml^{-1} piroxicam.

results [Figs 4(B) and 5(B)] showed that the peak height decreases as the reactor length increases. In both cases 70 cm reactor length (inner diameter 0.5 mm) was selected as this provided a high sampling frequency and reproducibility.

The effect of flow rate on peak height was studied over the range 0.5–2 ml min^{-1}. The results obtained are shown in Figs 4(C) and 5(C). A flow rate of 1.2 ml min^{-1} was selected.

From the results of the spectrometric studies, it is advisable to use a methanolic hydrochloric acid medium for the UV determination of piroxicam. Figure 6 shows the influence of the concentration of HCl on the peak height; maximum and constant peak heights are obtained at concentrations above 0.06 M HCl in methanol; 0.1 M HCl in methanol was selected as the medium.

When the formation of the Fe(III)–piroxicam complex was studied spectrophotometrically, it was observed that a methanolic medium was more suitable. The influence of the concentration of Fe(III) was studied in the range 1×10^{-4}–7.5×10^{-3} M with a fixed concentration of piroxicam of 200 μg ml^{-1} (6×10^{-4} M). Figure 7 shows that constant and maximum peak heights are obtained by a Fe(III) concentration higher than 2×10^{-3} M. A concentration of Fe(III) 5×10^{-3} M was selected, which is sufficient for the total formation of the complex in the range of the calibration graph used for the determination of piroxicam.

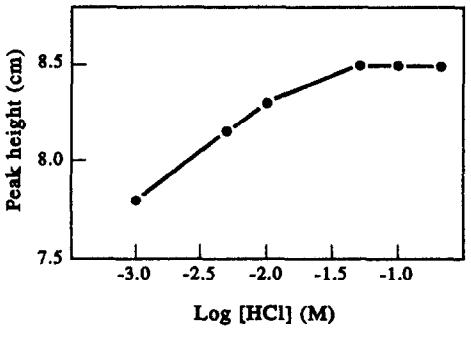

Figure 6
Effect of HCl concentration on peak height.

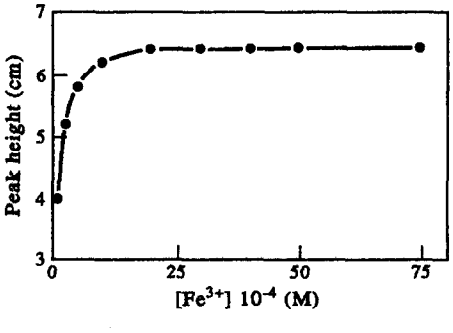

Figure 7
Effect of Fe(III) concentration of peak height.

Determination of piroxicam

A linear correlation was found between peak height and the concentration of piroxicam (Table 1). The lower limits of detection (signal-to-noise ratio = 3) were 0.15 μg ml^{-1} of piroxicam for the UV–FIA method and 7.5 μg ml^{-1} of piroxicam for the visible FIA method. The sampling frequency was 90 samples h^{-1}. The precision of the two methods was tested by analysing 10 replicate samples of 5 or 200 μg ml^{-1} of piroxicam; the relative standard deviations were 0.24 and 0.26%, respectively.

Table 1
Data for the calibration graphs (n = 7) for piroxicam using the proposed UV and visible FIA methods

	UV–FIA	vis–FIA
λ_{max} (nm)	332	520
Linear range (μg ml^{-1})	0.5–15	30–500
Intercept (mm)	3.94	0.67
Slope (mm μg^{-1} ml)	16.52	0.31
Correlation coefficient	0.9998	0.9994
Standard error of slope	1.60	1.76

Study of interference from other substances

The influence of frequently encountered excipients and additives in pharmaceutical dosage forms of piroxicam was investigated. The recovery results for the determination of 5 and 200 μg ml^{-1} of piroxicam are listed in Table 2. As can be seen, the proposed methods are sufficiently selective. However, interference from 5′-hydroxypiroxicam was observed for both methods.

Applications

The two proposed FIA methods were successfully applied to the analysis of different synthetic preparations that reproduced the compositions of the commercial formulations spiked with known amounts of the piroxicam in the range 10–70 μg and 0.5–2 mg, in order to obtain final solutions of 1–7 μg ml^{-1} and 50–200 μg ml^{-1}. The results show mean percentage recoveries of 100.9 ± 3.3 and 101.3 ± 2.4 for the UV–FIA method and vis–FIA method, respectively.

Table 2
Determination of piroxicam by the two methods in the presence of common excipients

	UV–FIA method			vis–FIA method		
	50 μg Piroxicam added			2 mg Piroxicam added		
Excipients	Mass* ratio	Found† (μg)	% Recovery ± SD	Mass* ratio	Found† (mg)	% Recovery ± SD
Saccharin	1	49.84	99.7 ± 0.88	5	2.05	102.6 ± 0.43
Lactose	15	49.57	99.2 ± 0.29	10	1.99	99.7 ± 0.43
Glycerol	25‡	50.78	101.6 ± 0.61	25‡	1.98	99.3 ± 0.76
Gelatin	25‡	50.40	100.8 ± 0.45	25‡	2.00	100.2 ± 0.65
Propylene glycol	15	49.69	99.3 ± 0.61	25‡	1.96	98.1 ± 0.70
Citrate	1	49.61	99.2 ± 0.48	25‡	2.02	101.2 ± 0.43
Glucose	20	49.68	99.4 ± 0.44	25‡	1.98	99.2 ± 0.55
Ethanol	25‡	49.21	98.4 ± 0.47	25‡	1.98	99.4 ± 0.65
Saccharose	1	49.29	98.6 ± 0.49	5	1.96	98.3 ± 0.34
Starch	25‡	49.44	98.9 ± 0.39	25‡	2.00	100.2 ± 0.34

* $W_{excipients}/W_{piroxicam}$.
† Mean of five determinations.
‡ Maximum mass ratio tested.

Table 3
Determination of piroxicam in pharmaceutical preparations

	Piroxicam content			Added piroxicam			
		Found*		UV–FIA method		vis–FIA method	
Sample	Stated	UV–FIA method	vis–FIA method	Added (mg)	Recovery (%)	Added (mg)	Recovery (%)
1	10†	10.26 ± 0.122	10.15 ± 0.043	5.0†	102.50	10.0	99.01
				10.0	99.34	15.0	100.38
				15.0	99.23	20.0	101.06
2	20†	19.93 ± 0.454	19.95 ± 0.140	10.0†	98.57	20.0	100.72
				20.0	101.32	30.0	100.48
				30.0	100.20	40.0	99.58
3	20†	20.32 ± 0.225	20.12 ± 0.070	10.0†	102.12	20.0	100.95
				20.0	100.53	30.0	99.04
				30.0	101.06	40.0	101.27
4	20‡	19.70 ± 0.294	19.70 ± 0.138	10.0‡	100.05	20.0	103.80
				20.0	98.55	30.0	101.24
				30.0	100.01	40.0	99.96
5	20§	19.80 ± 0.286	20.43 ± 0.134	10.0§	100.85	10.0	98.40
				20.0	99.89	15.0	99.20
				30.0	99.57	20.0	99.33
6	5‖	4.96 ± 0.052	5.06 ± 0.048	3.2‖	99.36	28.1	99.04
				6.3	99.68	42.1	99.10
				9.5	99.78	56.1	99.33
7	20¶	19.93 ± 0.389	19.98 ± 0.094	12.3¶	101.55	25.0	100.55
				24.6	99.81	37.8	100.36
				36.9	101.15	50.0	100.27

1, Doblexan capsules; 2, Improntal capsules; 3, Sasulen capsules; 4, Feldene tablets; 5, Feldene ampoules; 6, Improntal cream; 7, Feldene suppositories.
* Mean of five determinations ± SD. †mg capsule^{-1}. ‡mg tablet^{-1}. §mg ampoule^{-1}. ‖mg g^{-1} of cream. ¶mg suppository^{-1}.

The results obtained by the two methods were compared by applying the *F*-test and the *t*-test at the 95% confidence level. The calculated *F* and *t* values were 1.95 and 0.301, while the theoretical values were 5.82 and 2.177. This indicates that there is no significant difference between the two methods with respect to precision and accuracy.

Different pharmaceutical dosage forms of piroxicam were analysed by the proposed methods and the results are summarized in Table 3. For all the formulations examined the assay results were in good agreement with the declared content. The results obtained by the two methods were also compared by applying the *F*-test and *t*-test at the 95% confidence

level. The calculated F and t values did not exceed the theoretical values ($F = 9.60$; $t = 2.30$), indicating that there are no significant differences between the mean content of piroxicam obtained by the two proposed methods.

The validity of these methods was confirmed by applying the standard addition technique to the different pharmaceutical dosage forms of piroxicam analysed. The results are shown in Table 3.

Conclusions

The two proposed FIA methods for the determination of piroxicam have the advantage of being fast, simple, inexpensive and applicable over a wide concentration range with good precision.

Of the two methods proposed the UV–FIA is much more sensitive than the vis–FIA method. The first method allows the determination of piroxicam in a wider concentration range than the second. There is no significant difference between the two methods with respect to precision and accuracy.

The reported methods have been applied to the determination of piroxicam in pharmaceuticals, since there is no interference from common excipients that might be found in different formulations.

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