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Flow-injection spectrophotometric methods for the determination of tenoxicam

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Abstract

Two sensitive and rapid flow-injection spectrophotometric methods are proposed for the determination of tenoxicam (TX). In the first method, a Fe(III)–tenoxicam complex is formed in a methanolic medium and the absorbance is measured at 540 nm, while the second method involves measurement of the absorbance at 355 nm of a solution containing the drug in hydrochloric acid medium. In both methods, the peak heights were proportional to tenoxicam concentration over the ranges 7.0–320 and 0.5–8.5 mg l^{-1}, respectively. The methods have been applied to the routine determination of the drug in dosage forms.

Keywords: Tenoxicam; Fe(III); Flow-injection; Spectrophotometry; Pharmaceuticals

1. Introduction

Tenoxicam, 4-hydroxy-2-methyl-*N*-2-pyridinyl-2-H-thieno 2,3-e-1,2-thiazine-3-carboxamida-1,1-dioxide (TX), is a nonsteroidal anti-inflammatory drug (NSAID). It is a potent analgesic, anti-inflammatory and antipyretic agent, the effects of which are generally believed to be mediated by the inhibition of cyclooxygenase and subsequent prostaglandin formation. It is used in the treatment of patients with rheumatological disorders [1].

Several analytical methods have been described for the analysis of TX: electroanalytical [2–4], spectrophotometric [5–9] and chromatographic [10–15]. These methods have been applied to the determination of the drug in pharmaceuticals and biological fluids. Some of the methods suffer from interferences from the tablet matrix, whereas others are not suitable for routine analysis because they need sophisticated instruments not yet available in many control laboratories. An alternative simple chemical procedure for the determination of tenoxicam in its pure form and in pharmaceutical dosage is therefore necessary.

Flow-injection analysis (FI) is characterized by its simplicity, speed, the inexpensive equipment needed and the accuracy of its results. It is an important alternative to other analytical methods, with clear advantages in terms of the short time required for each assay. The usefulness of FI methods for routine analysis has been shown in a

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large number of determinations developed for clinical, pharmaceutical, food and environmental analysis. However, only one flow-injection method has been proposed for the determination of TX which is based on the reduction properties of the drug [16].

The object of the present work has been to study the complex formation reaction between tenoxicam and iron(III) and the development of two simple and fast FI-spectrophotometric methods for determination of tenoxicam. The proposed methods are based on the visible absorption of the orange-brown complex Fe(III)–TX in methanolic medium and on the visible absorption of tenoxicam in HCl medium. The FI methods introduced have been successfully applied to the determination of tenoxicam in pharmaceuticals.

2. Experimental

2.1. Apparatus

The FI system comprised a Gilson HP4 peristaltic pump with silicone flow tubes of 1.0 mm i.d. (Worthington, OH), an Omnifit injection valve (NY, USA), a Hellma 18-μl flow cell (Jamaica, NY) 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. An ultrasonic bath (Bransonic B5, 55 kHz, 14 W) was also used.

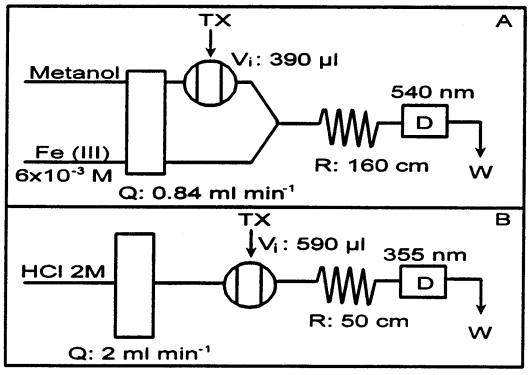

Fig. 1. FI manifolds for the determination of tenoxicam: Fe(III)–TX method (A); TX–HCl method (B).

2.2. Reagents

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

Tenoxicam stock standard solution (350 mg l^{-1} in methanol or 20 mg l^{-1} in 2 M HCl) was prepared by dissolving 35.0 or 2.0 mg of tenoxicam (Sigma, St Louis, MO) in 100 ml of methanol or in 100 ml of 2 M HCl respectively. Both stock standard solutions were stored in a refrigerator at approximately 4°C and remained stable for at least 1 month. Working standard solutions were prepared by suitable dilution of the stock standard solutions with methanol or 2 M HCl, respectively.

Iron(III) chloride solution (1.2×10^{-2} M) was prepared by dissolving 0.324 g of $FeCl_3.6H_2O$ (Merck) in 100 ml of methanol.

Hydrochloric acid (2 M) was also prepared.

2.3. Dosage forms of tenoxicam

(1) Artriunic tablets (Novag, Spain) and (2) Reutenox tablets (Solvay Pharma, Spain): tenoxicam 20 mg, lactose 90 mg, starch, talc, magnesium stearate, hydroxypropylmethylcellulose, titanium(IV) oxide and iron(III) oxide up to total tablet weight. (3) Tilcotil tablets (Roche, Spain): tenoxicam 20 mg with lactose and other excipients up to total tablet weight.

The average weight of each tablet for $n = 10$ of each of the pharmaceuticals was 203.4 ± 7.2 mg for artriunic, 207.1 ± 6.1 mg for reutenox and 202.2 ± 4.7 mg for tilcotil.

2.4. Recommended procedures for calibration

The flow-injection systems are shown in Fig. 1A,B. For the Fe(III)–TX procedure (Fig. 1A), 390-μl aliquots of tenoxicam solutions prepared in methanol at different concentrations (7.0–320 mg l^{-1}) were injected into an inert carrier stream of methanol. The solution of Fe(III) 6×10^{-3} M in methanol was mixed with the carrier stream at the down-stream confluence point. For the TX–HCl method (Fig. 1B), 590-μl aliquots samples of TX (0.5–8.5 mg l^{-1}) dissolved in 2 M HCl were

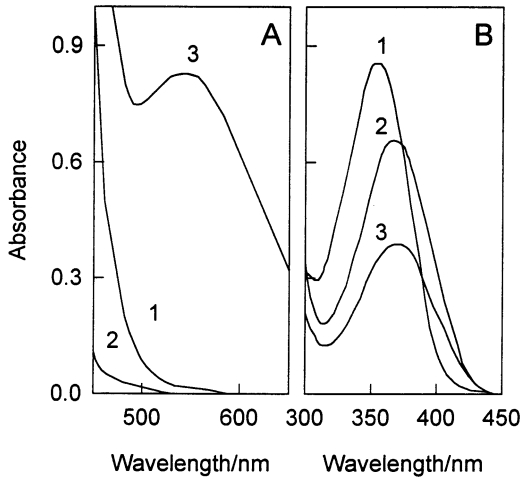

Fig. 2. (A) Absorption spectra of (1) 254 mg l^{-1} (7.5×10^{-4} M) tenoxicam in methanol; (2) 2.5×10^{-3} M Fe(III) in methanol; (3) 254 mg l^{-1} tenoxicam and 2.5×10^{-3} M Fe(III) in methanol. (B) Absorption spectra of 14.0 mg l^{-1} (4.1×10^{-5} M) tenoxicam in (1) 0.5 M HCl; (2) methanol; (3) 0.5 M NaOH.

injected into a 2 M HCl carrier stream. The absorbances were measured at 540 nm in the first method and 355 nm in the second. Calibration graphs were prepared by plotting the absorbances of the peak maximum versus tenoxicam concentrations.

2.5. Procedure for the assay of dosage forms

The average tablet weight was calculated from the contents of 10 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 10 mg of TX, was accurately weighed. The samples were shaken with 25 ml of methanol for the Fe(III)–TX method or 25 ml of 2 M HCl for the TX–HCl method. The mixtures were then introduced into an ultrasonic bath for 10 min, filtered through a Millipore filter and the filtrate was diluted with either methanol or 2 M HCl in a 100-ml calibrated flask. For the Fe(III)–TX method, aliquots of the methanolic solution obtained were injected in triplicate and the described calibration procedure was applied. For the TX–HCl method, 500-μl aliquots of the hydrochloric solution obtained were diluted to 10 ml with 2 M HCl and analyzed by the recommended procedure.

The procedure for validating the Fe(III)–TX method used aliquots of 1.0 ml methanolic solution of pharmaceutical sample equivalent to 100 mg l^{-1} of TX, to which were added different volumes (1.0–7.0 ml) of 35 mg l^{-1} TX methanolic solution. The mixtures were then diluted with methanol to 10.0 ml in a calibrated flask. The solutions obtained were analyzed by the recommended procedure. The TX–HCl method was validated using aliquots of 1.0 ml of 2 M hydrochloric solution of pharmaceutical sample equivalent to 100 mg l^{-1} of TX, to which were added different volumes (2.0–12.0 ml) of 20 mg l^{-1} of TX 2 M HCl solution. The mixtures were then diluted to 50.0 ml with 2 M HCl and the recommended procedure was applied.

3. Results and discussion

3.1. Preliminary studies

Tenoxicam reacts with iron(III) in methanolic medium to produce an orange-brown compound. Fig. 2A shows the absorption spectra of TX in methanol (curve 1), Fe(III) in methanol (curve 2) and TX in the presence of Fe(III) in methanol (curve 3). As can be seen, the third solution presents an absorption maximum at 540 nm, which is due to the formation of a Fe(III)–TX complex.

When the influence of acidity on the formation of this complex was studied, higher absorbance values were obtained with decreased acidity. The maximum absorbance was found in a methanolic medium, which was selected for subsequent studies.

The stoichiometry of the Fe(III)–TX complex was studied by applying the molar ratio and continuous variations methods. A stoichiometry 1:2 [Fe(III)]/[TX] was found with a molar absorptivity of 1.044×10^3 l mol^{-1} cm^{-1}.

The stability of the complex was studied immediately upon mixture of the reagents until 20 h later. The absorbance of the complex sharply increased up to 5 min and remained constant for 1 h. It then increased by 0.6 and 2.5% after 2 and 24 h, respectively.

The stability constant of the Fe(III)–TX complex was calculated and a value of $K = (1.63 \pm 0.29) \times 10^8$ was obtained.

The reaction between Fe(III) and TX was applied to developing a FI-spectrophotometric method for determining tenoxicam.

The absorption spectra of tenoxicam in hydrochloric acid (curve 1), methanol (curve 2) and sodium hydroxide (curve 3) are shown in Fig. 2B. As can be seen TX has a well defined absorption maximum at 355 nm in acidic medium and 372 nm in methanol or basic medium. Hydrochloric acid was found to give the highest sensitivity by enhancing the absorbance. The molar absorptivity for TX in a 2 M HCl medium was $\varepsilon = 3.193 \times 10^4$ l mol^{-1} cm^{-1}.

The measurements of the absorbance of tenoxicam in HCl at 355 nm were used to develop a FI-spectrophotometric method for determining the drug.

3.2. Flow systems

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configurations and the approximate ranges of the tested parameters. The design of the manifolds selected in both cases are shown in Fig. 1. For the Fe(III)–TX method, (Fig. 1A) a two-channel FI assembly was adopted, in which the sample was injected into the methanol stream, which is then mixed with a stream of Fe(III) dissolved in methanol. The reagents and the methanol carrier stream were pumped at the same flow rate to achieve effective mixing of the sample and reagent solutions. Fe(III) reacted with TX to produce a coloured compound and the absorbance was measured at 540 nm in the detector previously adjusted to zero with the Fe(III) carrier solution. The presence of the TX caused an increase in the analytical signal, which was proportional to its concentration.

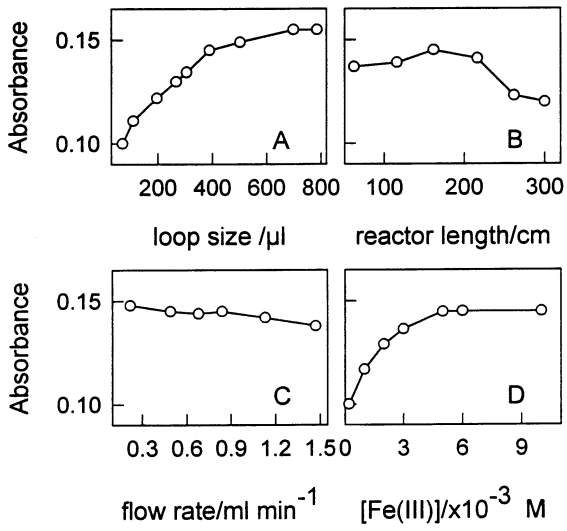

Fig. 3. Effect of the loop size (A); reactor length (B); flow rate (C) and Fe(III) concentration (D), on the peak height in the Fe(III)–TX method. Sample injected, 100 mg l^{-1} tenoxicam.

In the FI method based on the measurement of the absorbance of TX in HCl medium, (Fig. 1B) a one-channel FI assembly was selected. The TX sample dissolved in 2 M HCl was injected into a 2 M HCl stream and the absorbance was measured in the detector at 355 nm.

The use of FI as an alternative to existing methods for TX determination is dependent on optimization of the 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 best experimental conditions for operating the FI manifold. All the variables were selected by the univariate method.

Fig. 3 shows the effects of sample injection volume, reactor length and flow rate on the peak height of the Fe(III)–TX method. The volume of sample injected was varied from 70 to 780 µl by changing the length of the sample loop in the injection valve, while the other variables remained fixed (100-cm reactor length, 0.84 ml min^{-1} flow rate, 6×10^{-3} M Fe(III) solution and 100 µg ml^{-1} TX solution injected). The absorbance increased with increasing loop size (Fig. 3A). A loop size of 390 µl was chosen as a compromise between high sensitivity and low sample consumption.

The influence of reactor length was studied from the minimum distance possible between injection valve and detector, 0.5 m, up to 3.5 m in the same experimental conditions selected above. As can be seen from Fig. 3B, maximum ab-

Table 1
Data for the calibration graphs ($n = 12$) for tenoxicam using the proposed FI methods

Parameter	Fe(III)–TX method	TX–HCl method
λ_{max} (nm)	540	355
Linear range (mg l^{-1})	7.0–320	0.5–8.5
Slope (A l mg^{-1})	1.49×10^{-3}	5.32×10^{-2}
S.E. of slope ($\pm$)	4.3×10^{-6}	4.0×10^{-3}
Intercept	3.7×10^{-3}	5.6×10^{-3}
S.E. of intercept ($\pm$)	6.2×10^{-4}	2.7×10^{-3}
Correlation coefficient	0.9999	0.9996

sorbance values were obtained at 1.6–2.3 m. A 1.6-m reactor length (i.d. 0.5 mm) was selected as this provided the highest analytical signal and low residence time.

The effect of flow rate on peak height was studied over the range 0.2–1.5 ml min^{-1} and in the same experimental conditions. Fig. 3C shows that this variable had little influence on the absorbance in the studied range. A flow rate of 0.8 ml min^{-1} was selected, as a compromise between reproducibility and sampling rate.

The influence of the concentration of Fe(III) was studied in the range 1.7×10^{-4}–1.2×10^{-2} M with a fixed TX concentration of 100 mg l^{-1} (2.9×10^{-4} M) and the same physical variables selected above. As can be observed from Fig. 3D, constant and maximum absorbance values were obtained in the concentration range of 5.0×10^{-3}–1.2×10^{-2} M. A Fe(III) concentration of 6.0×10^{-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 TX.

Similar studies were carried out to select the loop size, reactor length, flow rate and the HCl concentration for determination of tenoxicam by TX–HCl method. In all the experiments 4.0 mg l^{-1} of TX solution was injected. The loop size was varied from 70 to 1000 μl, with a reactor length of 0.5 m, a flow rate of 1.9 ml min^{-1} and 2 M HCl concentration. An increase in loop size produced an increase in peak height, which reached a maximum and constant value at and above 590 μl. A loop size of 590 μl was chosen.

The influence of reactor length was studied over the range 0.5–3.5 m in the same experimental conditions mentioned above. The results showed a decrease in the peak height with increasing reactor length. Accordingly, 0.5-m reactor length was selected as this provided a high sampling frequency.

The effect of flow rate on peak height was studied over the range 0.5–3.0 ml min^{-1} and the same experimental conditions described above. An increase in flow rate increased the peak height to reach a maximum constant value at and above 2 ml min^{-1}. A flow rate of 2 ml min^{-1} was selected.

According to the results of the spectrophotometric studies (Fig. 2B), it is advisable to use a hydrochloric acid medium for the FI-spectrophotometric determination of TX. The influence of HCl concentration on the peak height was studied over the range 0.1–2.0 M, and the same physical conditions selected. Maximum and constant peak heights were obtained at 2 M HCl concentrations, and this concentration was selected.

The flow systems selected provided a sampling frequency of 40 or 100 samples h^{-1} for Fe(III)–TX or TX–HCl method, respectively.

3.3. Analytical characteristics of the methods

Under the conditions outlined above, a series of standard solutions was injected in triplicate to test the linearity of the calibration graphs. The analytical results obtained are shown in Table 1.

The limit of detection, calculated according to the recommendations of IUPAC [17], were ($n = 10$) 1.1 mg l^{-1} of TX (3.2×10^{-6} M) for the Fe(III)–TX method and 0.08 mg l^{-1} of TX (2.3×10^{-7} M) for the TX–HCl method.

The precision of the two methods was tested by analysing 10 replicate samples of 8.0 and 63.0 mg l^{-1} of TX (Fe(III)–TX method) or 0.64 and 6.4 mg l^{-1} of TX (TX–HCl method). The R.S.D. were ± 0.60 and ± 0.58 or ± 1.40 and ± 1.05%, respectively.

The between-day precision of the two methods was also determined by obtaining six calibration graphs on randomly selected days during the 15 days that the experiment lasted. The R.S.D. of the slopes obtained were 3.9 and 4.2% for the Fe(III)–TX and TX–HCl methods, respectively.

3.3.1. Study of interference from other substances

The influence of frequently encountered excipients and additives in pharmaceutical dosage forms of tenoxicam on the proposed methods was studied by adding different amounts of the possible interferents to samples containing 80.0 mg l^{-1} of TX in the case of Fe(III)–TX method or 5.0 mg l^{-1} in the TX–HCl method. The results are given in Table 2. The tolerance limit was taken as the concentration causing an error of not more than $\pm 3\%$ in the determination of the drug. In accordance with the average tablet weight calculated and the composition of the tablets described in Section 2, the lactose/tenoxicam mass ratio in the tablets was 4.5, which meant that the mass ratio for the other excipients was also 4.5, regardless of the individual proportions. As can be seen in Table 2, no interference was observed from the presence of lactose, glucose, citrate, saccharose, starch, talc, magnesium stearate, hydroxypropylmethylcellulose, titanium(IV) oxide or iron(III) oxide, even when mass ratios much greater than that contained in the pharmaceuticals assayed were used.

Table 2
Effects of various foreign species on the determination of tenoxicam

Foreign species	Maximum mass ratio[a] tolerated	
	Fe(III)–TX method	TX–HCl method
Lactose	50[b]	100[b]
Glucose, hydroxypropylmethylcellulose	30	50
Starch, citrate, saccharose	20	100
Magnesium stearate	10	100
Titanium(IV) oxide	5	30
Iron(III) oxide	25	30

[a] $W_{excipients}/W_{tenoxicam}$.
[b] Maximum mass ratio assayed.

Table 3
Determination of tenoxicam in pharmaceuticals[a]

Sample	Tenoxicam content (mg $tablet^{-1}$)		
	Labelled	Fe(III)–TX method	TX–HCl method
Artriunic	20	20.19 ± 0.48	20.15 ± 0.17
Reutenox	20	19.68 ± 0.44	19.72 ± 0.25
Tilcotil	20	19.61 ± 0.27	19.80 ± 0.37

[a] Values are the mean of five determinations $\pm$ S.D.

3.4. Applications

The two proposed FI methods where successfully applied to the analysis of different pharmaceutical dosage forms containing tenoxicam and the results are summarized in Table 3. When different pharmaceuticals of tenoxicam were analysed by the proposed methods, interference from the sample matrix posed no problems. For all the formulations examined both FI methods assay results were in good agreement with the declared content.

The results obtained by the two proposed methods were compared by applying the F-test and t-test at 95% confidence level. In no case did the calculated F- and t-values exceed the theoretical values ($F_{4,4} = 6.388$, $t_8 = 2.37$), confirming that there are no significant differences between the two proposed methods with respect to precision and accuracy in the determination of tenoxicam in pharmaceuticals.

The validity of the two methods was confirmed by applying the standard additions technique to the different pharmaceuticals of tenoxicam analysed following the procedure described in Section 2. The results obtained expressed in mg $tablet^{-1}$ of TX are shown in Table 4. In all cases, quantitative recoveries of between 99.3 and 101.9% were obtained for TX.

Table 4
Recoveries of tenoxicam from pharmaceuticals[a]

Sample	Tenoxicam			
	Fe(III)–TX method		TX–HCl method	
	Added (mg tablet^{-1})	% Recovery[b]	Added (mg tablet^{-1})	% Recovery[b]
Artriunic	8.12	99.8 ± 1.2	9.07	99.5 ± 0.8
	16.08	99.9 ± 0.5	20.16	99.7 ± 0.2
	45.92	99.4 ± 0.8	41.35	101 ± 0.4
Reutenox	9.02	99.8 ± 0.5	10.32	99.7 ± 1.1
	21.69	99.5 ± 0.9	33.31	100.4 ± 1.2
	43.39	100.7 ± 0.7	50.02	100.1 ± 0.6
Tilcotil	11.45	99.7 ± 1.1	19.12	99.8 ± 0.8
	33.97	100.0 ± 1.5	28.17	100.1 ± 0.9
	41.43	99.8 ± 0.8	45.32	99.3 ± 1.1

[a] Labelled content of tenoxicam: 20 mg tablet^{-1}.
[b] Mean of five determinations ± S.D.

4. Conclusions

In this paper, the complex Fe(III)–tenoxicam is studied for the first time. This metallic organic complex is very stable in methanolic medium and has spectrophotometric characteristics suitable for application to the determination of the drug.

The two flow-injection spectrophotometric methods proposed for the determination of tenoxicam in pure and pharmaceutical forms have the advantages associated with flow injection analysis: simplicity, speed, the use of inexpensive equipment and accuracy. The methods, therefore, are faster and simpler than most of the methods reported.

Of the two procedures proposed, the TX–HCl method is considerably more sensitive than the Fe(III)–TX method, although the latter allows tenoxicam to be determined in a wider concentration range. There is no significant difference between the two methods with respect to precision and accuracy.

The two methods are useful for the quality control and routine analysis of tenoxicam in pharmaceuticals since there is no interference from the common excipients that might be found in commercial preparations.

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