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Short communication

Spectrofluorometric determination of diclofenac in tablets and ointments

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1. Introduction

As other nonsteroidal anti-inflammatory drugs (NSAID), diclofenac [2-(2′,6′-dichloroanilino)-phenylacetic acid] is a fenamate derivative displaying anti-inflammatory, analgesic and antipyretic activity, showing a potent reversible inhibition of prostaglandin synthesis both in vivo and in vitro [1]. It is used in the treatment of many diseases, such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, nonarticular rheumatism and sport injuries [1]. The side effects that have been reported are gastrointestinal effects, headache, dizziness, skin rushes, edema and hepatic and renal damage. In some cases, gastric or intestinal ulcers, bleeding ulcers, heart failure and sight trouble could also be produced.

Different analytical methods have been reported in the scientific literature for the determination of diclofenac in plasma, urine, synovial fluid and pharmaceutical preparations, mainly involving spectrophotometry/colorimetry [2–9], chromatography [10–21] and selected ion monitoring [22]. As part of a program devoted to the development of simple methods for quality control in pharmaceuticals preparations [23,24], we report on the possibility of quantitating diclofenac in both tablets and ointments using spectrofluorometry. This method is rapid, selective and sensitive, and no interference from excipients or other drugs accompanying diclofenac in the studied commercial formulations has been observed. This is especially important in the case of cianocobalamine, which has been reported to be fluorescent [25].

The spectrofluorometric determination of diclofenac [2-(2′,6′-dichloroanilino)-phenylacetic acid] in pharmaceutical tablets and ointments is

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described. It involves excitation at 287 nm of an acid solution (HCl 0.01 N) of the drug, and measurement of the fluorescence intensity at 362 nm. The linear range is 0.2–5.0 mg l^{-1}. No interference is observed from the excipients or from other drugs which accompany diclofenac in certain formulations (paracetamol or cianocobalamine).

2. Experimental

2.1. Apparatus

All fluorescence measurements were done on a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a 150 W Xenon lamp, using 1.00 cm quartz cells. Experimental parameters were: slit width: 5.0 nm, $\lambda_{exc} = 287 \pm 3$ nm, $\lambda_{em} = 362 \pm 2$ nm.

Absorbance measurements were done on a Beckman DU 640 spectrophotometer using 1.00 cm quartz cells.

2.2. Reagents

A stock solution of analytical grade diclofenac (400 mg l^{-1}) was prepared by dissolving 20 mg of diclofenac with distilled water into a 50.00 ml volumetric flask, and then diluting with distilled water (1:10). This latter stock solution was finally diluted with HCl 0.01 N before the fluorescence measurements (see below).

2.2.1. Pharmaceutical preparations

Tablets and ointments were obtained from the following laboratories: Beta (Oxagelsic), Bristol-Meyer-Squibb (Vesalion), Novartis (Voltaren and Voltaren Emulgel), Merck (Damixa), and Bagó (Dioxaflex), and processed as described below.

2.3. Calibration curve

Solutions for the calibration curve were prepared by suitable dilution of the stock solution with HCl 0.01 N in a 50.00 ml volumetric flask. The concentration range was 0.2–5.0 mg l^{-1}. The fluorescence intensity was measured at ($\lambda_{em} = 362$ nm, irradiating at $\lambda_{exc} = 287$ nm. The equation for the calibration curve is: $I = A + BC$, where I is the fluorescence intensity (in arbitrary units). After least-squares linear fit of the fluorescence emission data (Table 1), we obtained $A = 5(3)$, $B = 41.2(9)$, C = concentration of diclofenac in mg l^{-1}, $r^2 = 0.995$, $n = 33$ (three replicates of 11 points). Calibration data and relative standard deviations (RSD %) are given in Table 1. The detection limit (DL, calculated as $3\sigma_A/B$), was 0.2 mg l^{-1}.

2.4. Procedure for unknown aqueous samples and pharmaceutical samples

Aqueous samples were prepared by conveniently diluting different amounts of the stock solution with HCl 0.01 N. For commercial tablets, an amount of triturated capsules containing 20 mg of diclofenac was weighed and placed into a 50.00 ml volumetric flask, dissolved with distilled water, sonicated for 20 min and filtered. Then 5.00 ml of the filtrate were placed into another 50.00 ml volumetric flask and diluted with distilled water. A final dilution with HCl 0.01 N was performed in order to obtain concentrations within the linear calibration range. Only in one case (Voltaren D), the triturated capsule was

Table 1
Calibration data for the spectrofluorometric determination of diclofenac

Diclofenac[a] (mg l^{-1})	Fluorescence intensity[b]	RSD (%)[c]
0.00	1.5	
0.50	24.8	9.0
1.00	45.1	4.2
1.50	71.3	2.3
2.00	95.1	1.5
2.50	105.9	1.3
3.00	123.3	1.2
3.50	146.0	1.1
4.00	175.1	1.1
4.50	194.3	1.2
5.00	205.4	1.3
	$r^2 = 0.995$	

[a] Averages of three determinations.
[b] Arbitrary units.
[c] RSD (%) of each point from the regressed line.

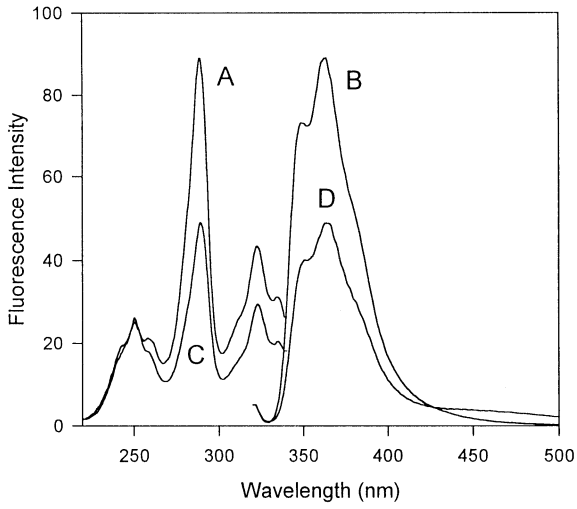

Fig. 1. Fluorescence spectra of aqueous solutions of diclofenac at different pH values: (A) Excitation, pH, 2; (B) emission, pH, 2; (C) excitation, pH, 12; and (D) emission, pH, 12. In all cases, the concentration was 2.00 mg l^{-1}. For recording emission spectra λ_{exc}, 287 nm; for excitation, λ_{em}, 362 nm.

weighed, dissolved in water but neither sonicated nor filtered. In all cases it was assumed that the actual content of the tablet corresponds to that reported by the manufacturing laboratories. A similar procedure to that described above was applied to the ointment.

3. Results and discussion

Diclofenac is soluble in water and emits fluorescence at 362 ± 2 nm when excited at 287 ± 3 nm (Fig. 1). The spectral characteristics are almost independent of the solution pH. However, significant changes of the fluorescence intensity as a function of pH were observed (Fig. 1). The plot of fluorescence intensity as a function of pH is a sigmoid with an inflection point near pH approximately 3, as expected from the equilibrium between the protonated and deprotonated forms of diclofenac. This previous study indicated that suitable conditions for the analytical determination of diclofenac involve maintaining the pH in the range 1–2.

On the other hand, the electronic absorption spectrum of diclofenac at pH 2 provides information as to the maximum concentration at which fluorescence linearity may be expected, i.e. that for which $A = 0.05$. This limit was estimated at approximately 1.8 mg l^{-1} however, in subsequent fluorescence experiments, the calibration plot was found to be linear in the range 0.2–5.0 mg l^{-1} (see Section 2). Notice that the absorption maximum of diclofenac in acid solution lies at 275 nm, whereas the excitation maximum appears at 287 nm (Fig. 1).

Unknown aqueous samples of diclofenac were studied by the above procedure (Table 2). The method was extended to several pharmaceutical preparations (five tablets and one ointment), with the results summarised in Table 3. It may be noticed that in some of the cases shown in Table 3, diclofenac is combined with other drugs, yet no interference is observed. Spectra of mixtures of pure diclofenac with paracetamol, cianocobalamine or betametasone are indistinguishable from that corresponding to the pure drug at the same concentration. This is especially important in the case of cianocobalamine, which has been reported to be fluorescent in acid media [25]. Likewise, no interference from the preparation excipients were observed. All recoveries are within the limits recommended by Pharmacopeia (i.e. between 90 and 110% of the declared amount) [26].

In conclusion, it has been shown that diclofenac can be determined in pharmaceutical preparations using a rapid, sensitive and selective spectrofluorometric method.

Table 2
Determination of diclofenac in synthetic samples

Taken	Found[a]	Recovery (%)	RSD (%)
1.50	1.52	102	5.3
2.50	2.42	97	3.5
3.00	2.71	91	2.2
3.50	3.55	101	3.4
4.00	4.11	103	2.6
5.00	5.18	104	3.8

[a] Average of three determinations.

Table 3
Determination of diclofenac in pharmaceutical preparations (tablets and ointment)

Preparation	Composition	Found[a] (mg per tablet) (Recovery %)
Dioxaflex (tablet)	Diclofenac (sodium salt) 75 mg	74 ± 1
	Excipients	(98)
Vesalion (tablet)	Diclofenac (potassium salt) 50 mg	53 ± 1
	Betametasone 0.30 mg	(107)
	Cianocobalamine 5 mg	
	Excipients	
Oxagelsic (tablet)	Diclofenac (potassium salt) 50 mg	52.5 ± 0.5
	Paracetamol 300 mg	(105)
	Excipients	
Damixa (tablet)	Diclofenac (potassium salt) 50 mg	50.0 ± 0.5
	Excipients	(100)
Voltaren (tablet)	Diclofenac (sodium salt) 50 mg	47.5 ± 0.5
	Excipients	(95.0)
Voltaren emulgel	Diclofenac sodium 1 g (% P/P)	1.09 ± 0.02% P/P
(ointment)	Excipients	(109)

[a] Average of three determinations ± SD. Recoveries were calculated on the assumption that the tablets contained the amount reported by the manufacturing laboratories. All values are given in mg per tablet, except in the case of the ointment.

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