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Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1331–1337

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Europium(III) ion probe spectrofluorometric determination of diclofenac sodium

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Received for review 4 December 1994; revised manuscript received 15 March 1995

Abstract

A new method has been devised for the determination of diclofenac sodium in bulk and in pharmaceutical preparations using Eu^{3+} ions as the Fluorescent probe. The technique was built around the hypersensitive property of the transitions of the fluorescent probe ion, Eu^{3+}, at 616 nm. This is normally a forbidden transition, but the interaction with diclofenac sodium, which contains a carboxylic group, makes the transition allowed and enhances the intensity of its fluorescence emission. The Eu^{3+} fluorescence emission at 592 nm comes from a non-hypersensitive transition and is not affected by ligation. The intensity ratio, R, defined as I_{592}/I_{616}, was used as a measure of the percentage of bound probe ions. Diclofenac and Eu(III) forms a (1:1) molar complex.

The relative stability constant of the complex was found to be 10^5. A linear relationship between bound Eu^{3+} and the concentration of diclofenac sodium was found for concentrations from 10 to 200 $\mu g\ ml^{-1}$, with a recovery percentage of 100.22 ± 2.27. The method shows a good agreement with a spectrophotometric method.

Keywords: Diclofenac sodium; Europium(III) ions; Pharmaceutical; Probe spectrofluorometry

1. Introduction

Diclofenac sodium, sodium (2,(2,6-dichloroanilino)phenyl), acetate has been shown to be an effective and well tolerated non-steroidal anti-inflammatory agent for the treatment of rheumatoid arthritis [1]. The most commonly used analytical techniques are ultraviolet spectrophotometry [2], colorimetry [3], GC [4], HPLC [5], TLC [6] and NMR [7].

Lanthanide ion probe spectrofluorometery (LIPS) introduced by Horrocks and Sudnick [8] employs two emission lines of Eu^{3+} located at 592 nm and 616 nm. The 616 nm emission line is produced by a hypersensitive transition [9], $^5D_0 \rightarrow {}^7F_2$, which is normally a forbidden transition, but interaction with different ligands makes the transition allowed and enhances the intensity of its emission [10].

The use of lanthanide ions as florescent probes has been well documented [11]. The europium metal ion has been the lanthanide of choice in most applications and its spectral features are widely documented [12]. Moreover, Eu^{3+} has been used for the determination of theophylline in the presence of caffeine [13] applying an energy transfer technique. In this paper, we describe a spectroscopic technique for the determination of diclofenac sodium using LIPS.

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SSDI 0731-7085(95)01567-1

2. Experimental

2.1. Apparatus

A Lambda Physik EMG 102 XeCl excimer laser and a FL 3002 tunable dye laser were used in tandem to supply a high intensity tunable source. The average output of the XeCl excimer was 1.6 W (10 Hz repetition rate) at 308 nm. A tunable ultraviolet emitting dye laser was used to provide an excitation source of 394.3 nm, which corresponds to a resonant absorption transition of the Eu metal ions. The Eu florescence was collected and frequency analyzed with a Spex 1877 triple monochrometer. The fluorescence was detected by a charge coupled detector (ccd 9000 photometric Ltd). The recorded curves were fitted with Lab Calc (Galactic Software) using a Gaussian model. The areas under the two peaks at 592 nm and 616 nm were integrated and the ratio of the two areas was taken as the R value of the sample.

2.2. Materials

Diclofenac sodium and europium(III) chloride were from Aldrich; hydrochloric acid and p-dioxane were Baker analyzed reagents.

2.3. Reagents

A standard diclofenac sodium solution (1000 $\mu g\ ml^{-1}$) was prepared in deionized water and its pH was adjusted to 5.5 using dilute hydrochloric acid solution. A 2×10^{-3} M europium chloride solution was prepared by dissolving 0.7328 g europium chloride (molecular weight = 258.32) in 1 l deionized water (pH 5.5).

2.4. General procedure

An aliquot volume of standard diclofenac sodium solution equivalent to 100–2000 μg was transferred into a 10 ml calibration flask, and 2 ml of p-dioxane and 0.5 ml of europium chloride solution were added. The solution was mixed well and brought to volume with deionized water. The fluorescence was measured from 592 to 616 nm (excitation at 394.3 nm). The curves were fitted using a gaussian model and ratio was calculated from the fitted peak areas.

2.5. Procedure for determination of diclofenac sodium in pharmaceutical preparations

Voltaren tablets and rheumafen capsules

Twenty tablets or the mix contents of ten capsules were weighed and powdered. To the quantity of powder equivalent to 50 mg of diclofenac sodium was added 20 ml of methanol. This was filtered into a suitable flask, the powder washed three times with 10 ml methanol, filtered, and the combined filtrate mixed well. Methanol was evaporated under nitrogen gas to dryness. The residue was dissolved in 50 ml water (pH 5.5). The solution was transferred into a 100 ml calibrated flask and brought to volume with deionized water. 2 ml of this solution was assayed as described in Section 2.4.

Voltaren ampoule, 75 mg (3 ml)$^{-1}$

The contents of five ampoules were mixed. 2 ml was evaporated under nitrogen gas to dryness. 20 ml methanol was added to the residue. The procedure was completed as described above.

Voltaren suppositories and ointment

The contents of either five suppositories or two bottles of ointment were weighed and mixed well. To the quantity of suppositories or ointment equivalent to 50 mg diclofenac sodium was added 20 ml of methanol. The procedure was then completed as described above.

3. Results and discussion

3.1. Lanthanide ion probes

The most fundamental and necessary characteristic of any probe in a study of this kind is the ability to determine quantitatively the concentration of the free and bound metal simultaneously [14]. This information is uniquely encoded in the experimental Eu^{3+} profiles. Fig. 1 illustrates the ratio of the Eu^{3+} emission peaks of solutions containing equal amounts of diclofenac sodium, but different concentrations of total Eu^{3+}; 0.5 ml of 2×10^{3} M was the optimum volume for such study in a total volume of 10 ml. Fig. 2 shows the fluorescence spectra of Eu^{3+} ions in the absence and presence of diclofenac sodium.

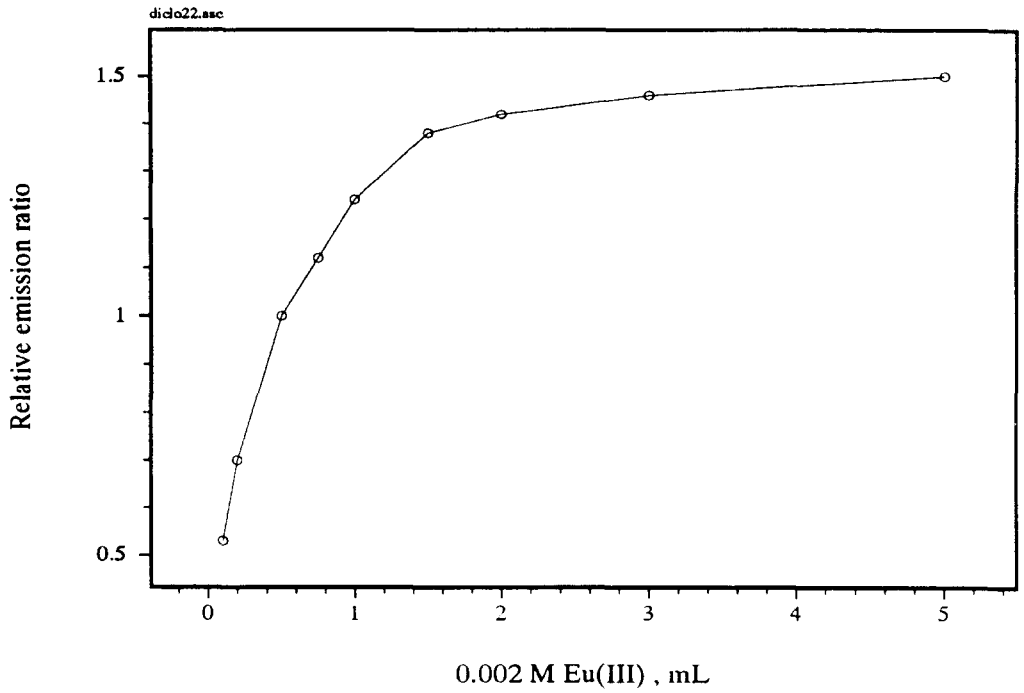

Fig. 1. Effect of Eu^{3+} ions on the fluorescence ratio of 0.0001 M diclofenac sodium; experimental error is ± 5%.

3.2. Effect of organic solvents

The europium diclofenac complex was insoluble in aqueous solution, so many organic miscible solvents such as ethanol, methanol, acetonitrile and *p*-dioxane were examined. *p*-Dioxane was the solvent chosen. 3 ml of *p*-dioxane in a total volume of 10 ml was sufficient to produce a clear solution and did not interfere with the measurements. Moreover, the complex was extractable with ether, but the free Eu ions were not, so ether was not suitable in our studies.

3.3. Effect of buffer and pH

Acidic buffers such as acetate buffer, MOPS buffer, MES buffer, THAM buffer and phosphate buffer were examined, and the results showed that these buffers were bound to Eu^{3+} ions, especially in the presence of dioxane, and interfered with the measurements. The adjustment of pH is important, since Eu^{3+} forms a Eu $(OH)^{2+}$ species in neutral solutions and at pH < 4 the binding of Eu with diclofenac sodium is decreased. The pH of the final solution must be kept between 5 and 6. Adjusting the initial pH of the Eu^{3+} and diclofenac sodium solutions to pH 5.5 using dilute hydrochloric acid solution kept the pH of the mixture between 5 and 6.

3.4. Effect of time and temperature

The reaction of the Eu^{3+} ion with diclofenac sodium was almost instantaneous, producing a white precipitate. To prevent precipitation, *p*-dioxane was added before the Eu^{3+} ions. A stable ratio was produced within 5 min and the complex was stable for at least 24 h. Temperature had little effect on complex formation and stability, so the reaction was carried out at ambient temperature.

3.5. Molar ratio

Fig. 3 shows the spectroscopic titration of 1 ml of 2×10^{-3} M europium chloride with a 2×10^{-1} M solution of diclofenac sodium. It was found that the complex behaves as a 1:1 molar complex.

3.6. Performance characteristics

The basis of the experimental determination of diclofenac sodium with Eu^{3+} ions lies in the existence of a hypersensitive transition of the Eu^{3+} ion, $^5D_0 \rightarrow {}^7F_2$ (616 nm). Hypersensitive emissive transitions are specific absorption or emission transitions of the lanthanide ions that are extremely sensitive to ligation. The intensity of a hypersensitive transition is enhanced in the metal ligand complex relative to that

transition in aquo ions [14]. Experimentally, this is observed by making the concentration of Eu^{3+} small relative to the amount of diclofenac sodium, such that the majority of the metal ion is complexed with the ligand. Such observations have been previously reported [14] for Eu^{3+} ions with humic acid. The effect of the hypersensitive transition is most obvious from the observation of several emission bands of Eu^{3+} ions. Fig. 2 illustrates the emission spectrum of the 1×10^{-4} M Eu^{3+} ion in the presence of different concentrations of diclofenac sodium.

Diclofenac sodium has a single binding site (through a carboxylic group) and the stoichiometry of the raction appears to be 1:1. The reaction of diclofenac sodium with the Eu^{3+} ion can be represented as [14]

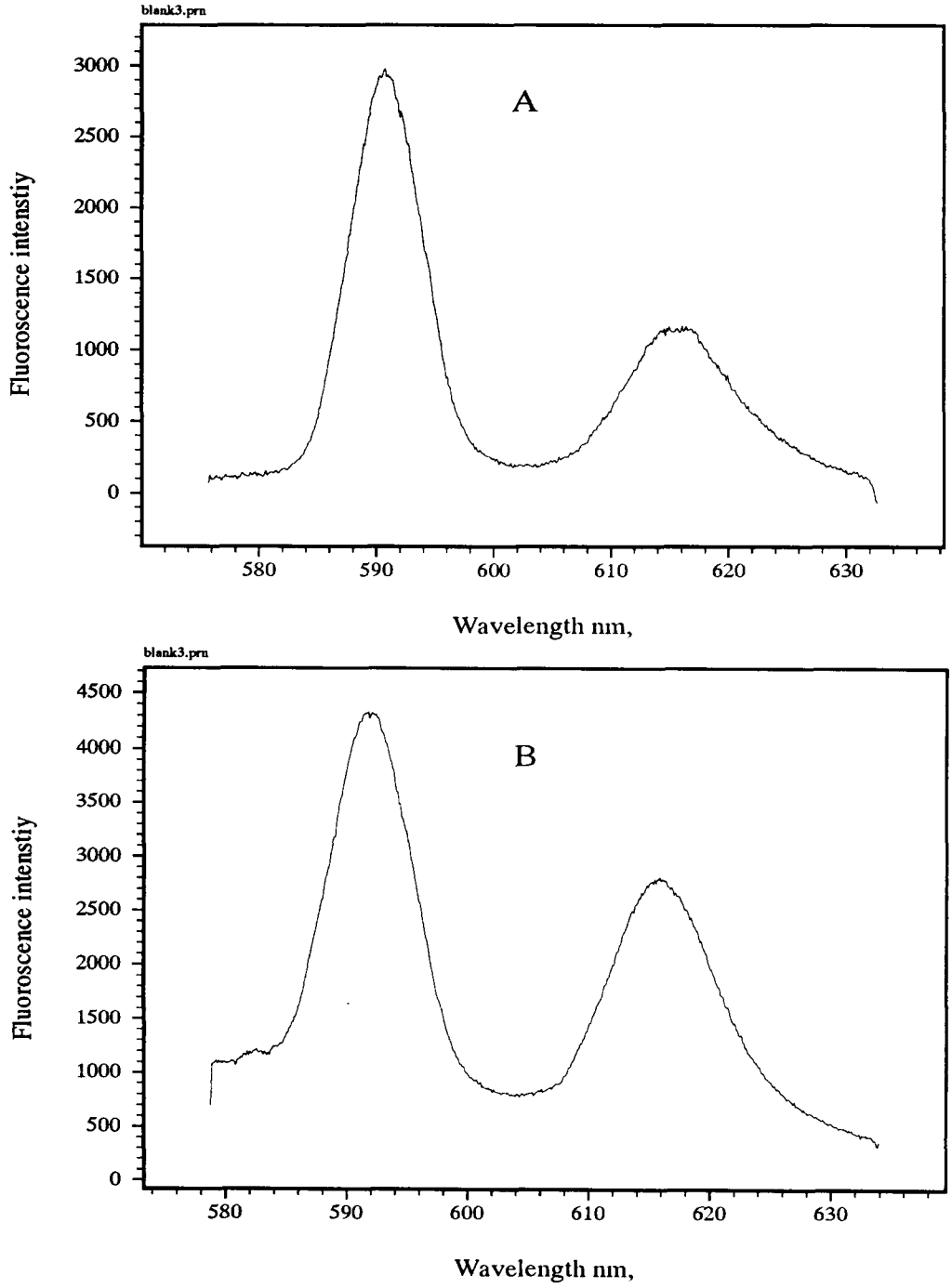

Fig. 2 (A and B)

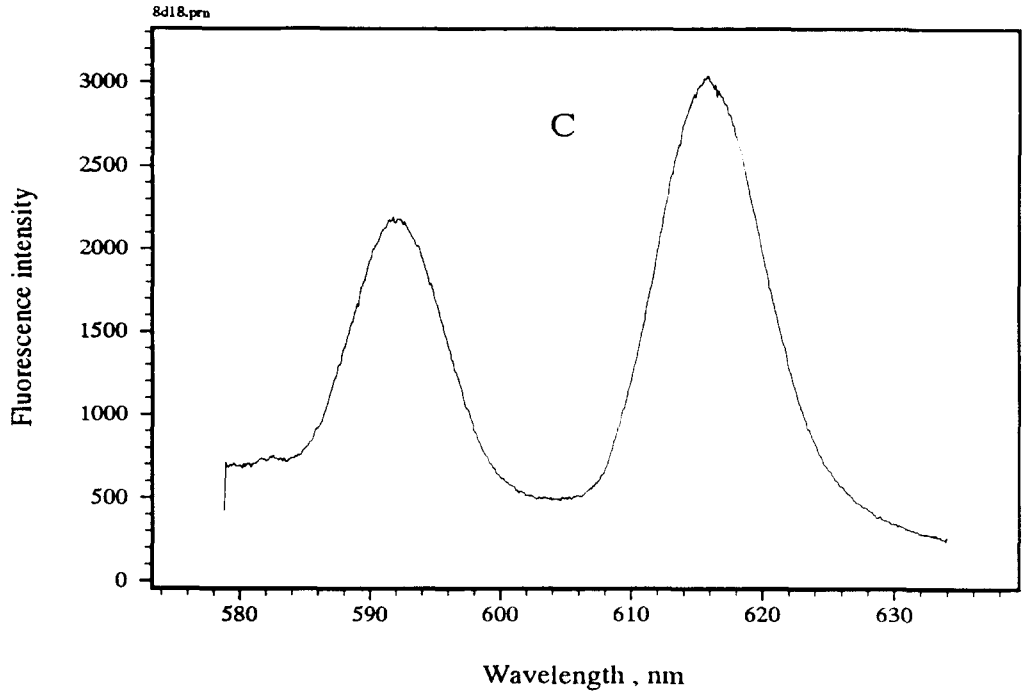

Fig. 2. Emission spectrum of 0.5 ml of 0.002 M Eu^{3+} (A) without diclofenac sodium, (B) in the presence of 100 μg ml^{-1} diclofenac sodium and (C) in the presence of 200 μg ml^{-1} diclofenac sodium.

$$M + L \rightarrow ML \quad (1)$$

where M is the metal ion, L as the ligand and ML is the complex product. The binding constant K for the reaction is [14]

$$K = \frac{[ML]}{[M][L]} \quad (2)$$

where [ML] is the concentration of the metal complex, [M] is the concentration of free metal, and [L] is the concentration of ligand. K was found to be 10^5.

The free Eu ion concentration was calculated by [14]

$$[M] = \frac{C_M L_B (R - L_S)}{R(L_B - L_S)} \quad (3)$$

where [M] is the concentration of free metal ions, C_M is the total concentration of metal added, L_B is taken to be the ratio of the Eu^{3+} peaks in the absence of diclofenac sodium, and L_S is taken to be the ratio when the Eu^{3+} concentration is very small with respect to a large ligand concentration. In this study, the values of L_B and L_S were measured to be 2.04 and 0.33, respectively. R is the ratio of I_{592}/I_{616} of the bound and free metal species.

The bound Eu^{3+} ion concentration was calculated as [14]

$$[ML] = C_M - [M] \quad (4)$$

A calibration line was calculated by plotting [ML] against the concentration of diclofenac sodium. It was found that there is a linear relationship between the concentration of diclofenac sodium and concentration of bound Eu^{3+} ions within the concentration range 10–200 μg ml^{-1}, with a standard deviation of 2.27 μg ml^{-1}.

Table 1 shows the results of the assay of diclofenac sodium by the proposed method and a reference spectrophotometric method. The proposed method has proved to be accurate and precise. The major advantages of using Eu^{3+} chelate as fluorescent probes are the high quantum yield (the euopium chelate quantum yield reaches up to 0.18 [15]), an exceptionally large Stoke's shift, a narrow emission peak, and optimal emission and excitation wavelengths for use in biological and pharmaceutical materials [16].

Table 2 shows the results of the assay of some pharmaceutical preparations containing diclofenac sodium. Ingredients other than diclofenac sodium, such as diluents of tablets ointments and injectables, were found not to interfere when the proposed procedure was used. The organic dyes that coated the Voltaren tablets were found to be insoluble in methanol, so the active ingredient was first extracted in methanol as described in the Ex-

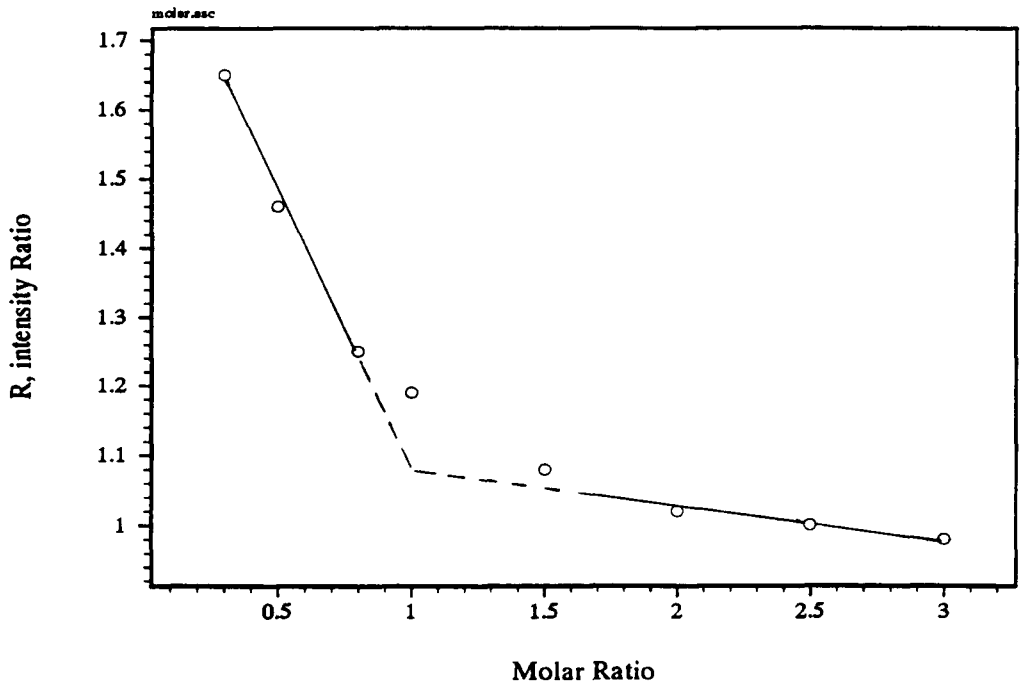

Fig. 3. Spectroscopic titration of 1 ml of 0.002 M Eu^{3+} ions with 0.002 M diclofenac sodium solution; experimental error is ±5%.

Table 1
Assay results of pure diclofenac sodium by the proposed method and a reference method [a]

Method	Calibration plot	r	% Recovery[b] ± SD
Proposed	$[M_L] = 0.3098\ E^{-6}\ C_M - 0.3099\ E^{-5}$	0.9997	100.2 ± 2.3
Reference	$A = 0.04058\ C_M - 0.3986\ E^{-2}$	0.9988	100.4 ± 2.9

$[M_L]$ is the concentration of bound Eu^{3+}, C_M is the concentration of diclofenac sodium, A is the absorbance of diclofenac sodium at 283 nm in methanol, and r is the correlation coefficient.
[a] Reference method is direct spectrophotometric method in methanol at 283 nm[2].
[b] Average of at least eight duplicate determinations.

Table 2
Assay results of diclofenac sodium in pharmaceutical preparations by the proposed method and a spectrophotometric method [2]

Pharmaceutical preparation	Labelled amount (mg)	% Recovery [a] ± SD	
		Proposed method	Reference method
Tab. [b]	25/tab.	105.4 ± 2.8	100.0 ± 1.1
Cap. [c]	100/cap.	103.3 ± 1.8	99.9 ± 2.1
Amp. [b]	25/ml	100.2 ± 1.8	101.4 ± 1.6
Sups. [b]	100/sup.	105.5 ± 2.0	102.5 ± 0.8
Oint. [b]	10/g	103.83 ± 0.2	101.2 ± 1.7

[a] Average of at least three triplicate determinations.
[b] Voltaren, Ciba Giegy, Egypt.
[c] Rheumafen, Apic Co., Egypt.

perimental section. The proposed procedure has proved to be suitable for routine analysis and quality control of diclofenac sodium in pharmaceutical preparations.

4. Conclusion

Eu^{3+} as a probe ion in a spectrofluorometric technique has been applied to the determina-

tion of diclofenac sodium in the pure form and in pharmaceutical preparations. This is the first time such a technique has been adapted for pharmaceutical quality assurance of diclofenac sodium.

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