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A Simple Spectrofluorimetric Method for Determination of Piroxicam and Propranolol in Pharmaceutical Preparations

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ABSTRACT

A new, simple, sensitive and rapid spectrofluorimetric method for the determination of piroxicam and propranolol in pharmaceutical formulations has been described. The method is based on the oxidation of piroxicam and propranolol with cerium (IV) to produce cerium (III), whose fluorescence was monitored at 352 nm while excited at 255 nm. The variables affecting oxidation of these drugs were studied and optimized. Under the experimental conditions, the calibration graphs were linear over the range of 0.02-3.0 and 0.02-2.4 mg/L, respectively, for piroxicam and propranolol. The limit of detection for piroxicam and propranolol was 0.006 and 0.008 mg/L, respectively, and the relative standard deviation of 5 replicate determinations of these drugs at 1.0 mg/L concentration level was 1.65 and 1.79%, respectively. Good recoveries in the range of 95-108% were obtained for spiked samples. The proposed methods were successfully applied to the determination of piroxicam and propranolol in commercial pharmaceutical formulations.

Key words: piroxicam, propranolol, spectrofluorimetry, cerium

INTRODUCTION

Piroxicam [4-hydroxy-2-methyl-n-(2-pyridyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide] is a non-steroidal anti-inflammatory drug with analgesic and anti-pyretic activities. Piroxicam exhibited a rapid and effective response in the treatment of many diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout juvenile rheumatoid arthritis, musculoskeletal disorders, postpartum pain and sport injuries. The most serious reported side effects are gastrointestinal effects, such as ulcer, bleeding ulcers, etc[1].

Propranolol (1-isopropylamino-3-(1-naphthyloxy)-2-propranolol) is a β-adrenergic blocking drug that has been widely applied to the treatment of cardiac arrhythmia, sinus tachycardia, angina pectoris and hypertension. It has also been suggested to use in numerous conditions including dysfunction labour and anxiety[2]. Propranolol is also used in low activity sports, reducing cardiac frequency, contraction force and coronary flow. Therefore, it has been included in the list of forbidden substances by the International Olympic Committee. Accordingly, the development of rapid and direct monitoring strategy of propranolol calls for interest[3].

Several analytical methods have been proposed for the determination of piroxicam in pharmaceuticals, namely spectrophotometric[4-8], chromatographic[7-9] and electrochemical techniques[10-12], with special attention to those using spectrofluorimetry[1,13-17].

Propranolol has been determined in pharmaceutical preparations by a range of methods, such as fluorimetry[18-20], phosphorimetry[21,22], chemiluminescene[23,24], spectrophotometry[25,26], atomic absorption[27,28], electrochemical[2], chromatography[29] and electrophoresis[30]. USP pharmacopoeia describes chromatographic assay for the determination of both piroxicam and propranolol[31].

As fluorescence spectrometry with great sensitivity and selectivity as well as relatively low cost for the operation, is widely used in quantitative analysis of pharmaceuticals, we propose here a simple and inexpensive spectrofluorimetric method for the determination of piroxicam and propranolol in pharmaceutical preparations. The method involves the oxidation of piroxicam or propranolol with Ce(IV) and subsequent monitoring of the fluorescence of Ce(III) at 352 nm after excitation at 255 nm.

$$\text{Piroxicam or Propranolol} + \text{Ce(IV)} \xrightarrow{H_2SO_4} \underset{\text{(Fluorescent)}}{\text{Ce(III)}}$$

MATERIALS AND METHODS

I. *Apparatus*

A Shimadzu RF-5301 PC spectrofluorophotometer, equipped with a 150 W Xenon lamp and 1.00 cm quartz

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cells, was used for the fluorescence measurements. Both excitation and emission slits were adjusted to 3 nm.

II. *Reagents*

Five hundred mg/L solutions of piroxicam (from Zahravi, Tabriz, Iran; purity of 99%) and propranolol (from Darupakhsh, Tehran, Iran; purity of 99%) were prepared by dissolving appropriate amount of drugs in 10.0-mL ethanol and 0.5-mL NaOH or HCl (1.0 M), in the case of piroxicam or propranolol, respectively, and diluting to 25 mL with water. These solutions were protected from the light and kept at 4°C for two week. Working standard solutions were prepared by appropriate dilution of these stock standard solutions. The Ce(IV) solution at concentration of 2.5 mM was prepared from Ce(IV)-sulfate-tetrahydrat (E-Merck) in 0.2 M sulphuric acid and was kept at 4°C for two week. A 5.0 M H_2SO_4 solution and 1.0 M NaOH or HCl solutions were also prepared.

All other reagents were of analytical reagent grade (E. Merck) and all solutions were prepared in doubly distilled water.

III. *Recommended Procedures for Calibration*

Aliquots of 0.02-3.0 mL (or 0.02-2.4 mL) from 10 mg/L piroxicam (or propranolol) standard solution were transferred into a series of 10-mL volumetric flasks. Point two (or 0.1) mL Ce(IV) and 1.0 mL H_2SO_4 solution were then added to each flask successively. Each flask was made up to the volume with water and the solutions were allowed to stand at room temperature for 10 min. The fluorescence intensity of each solution was measured at 352 nm while excited at 255 nm against a blank prepared similarly.

IV. *Procedure for the Pharmaceutical Preparations*

The contents of five capsules of piroxicam (Razak, Tehran, Iran), each containing 10 mg piroxicam, were transferred to a 100-mL volumetric flask and dissolved in 15-mL ethanol and 2.0-mL NaOH solution. Then, the volume was adjusted to the mark with water to obtain a 500 mg/L solution of piroxicam.

In the case of piroxicam gel (Hakim, Tehran, Iran), containing 0.5 g piroxicam per 100 g gel, sample containing 2.5 mg piroxicam was weighed into a 50-mL beaker and dissolved in 15-mL ethanol and 2.0-mL NaOH solution. It was then filtered into a 50-mL volumetric flask and diluted to the mark with water. Thus, a 50 mg/L solution of piroxicam was obtained.

In the case of propranolol tablets (Tolidaru, Tehran, Iran), contents of ten tablets, each containing 10 mg propranolol, were accurately weighed individually and finely powdered. Powdered sample containing 5 mg propranolol was weighed and placed into a 15-mL glass tube, dissolved in 10-mL ethanol and 1.0-mL HCl solution and was vigorously shaken on a vortex mixer for 30 sec. The solution was then filtered and transferred into a 100-mL volumetric flask. The residue was washed in enough ethanol and the solution was finally made up to the mark with water. Thus, a 50 mg/L solution of propranolol was obtained. These solutions were diluted quantitatively to yield concentrations in the range of working standard solution and then the piroxicam or propranolol contents were determined by the procedures proposed above.

RESULTS AND DISCUSSION

Ce(IV) serves as an oxidizing agent for the determination of drugs by monitoring the fluorescence of the reduced Ce(III)[32-35]. Ce(III) is usually more fluorescent than formed product and therefore, measurement of its fluorescence can be employed as a sensitive method for the determination of certain drugs. In the present work piroxicam or propranolol were oxidized by Ce(IV) in sulphuric acid medium and the fluorescence intensity of the produced Ce(III) was monitored at 352 ± 3 nm after excitation at 255 ± 3 nm. Excitation and emission spectra for piroxicam and propranolol systems are given in Figures 1 and 2, respectively.

I. *Effect of Ce(IV) Concentration*

The effect of Ce(IV) concentration on the fluorescence intensity was evaluated in the range of 0.1 – 2.5×10^{-4} M. In Figure 3, it was shown that Ce(IV) at concentrations of 2.5 – 5.0×10^{-5} and 1.25 – 2.5×10^{-5} M lead to the saturation signals in the case of piroxicam and propranolol, respectively. At concentrations lower than this range the fluorescence intensity dropped due

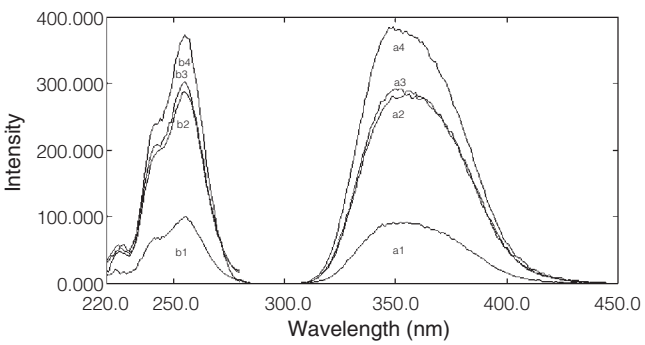

Figure 1. Emission and excitation spectra: a1 & b1: emission and excitation of Ce(III) in reagents blank; a2 & b2: emission and excitation of Ce(III) after oxidation with 0.4 mg/L piroxicam solution (prepared from piroxicam capsule); a3 & b3: emission and excitation of Ce(III) after oxidation with 0.4 mg/L piroxicam solution (prepared from piroxicam gel); a4 & b4: emission and excitation of Ce(III) after oxidation with standard solution of piroxicam (0.5 mg/L); 5.0×10^{-5} M Ce(IV); 0.5 M H_2SO_4.

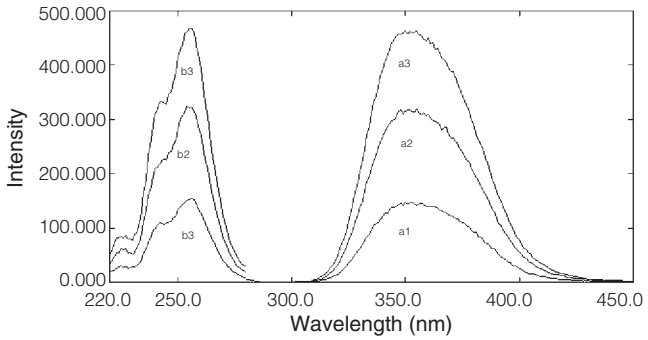

Figure 2. Emission and excitation spectra: a1 & b1: emission and excitation of Ce(III) in reagents blank; a2 & b2: emission and excitation of Ce(III) after oxidation with 0.4 mg/L propranolol solution (prepared from propranolol tablet); a3 & b3: emission and excitation of Ce(III) after oxidation with standard solution of propranolol (0.6 mg/L); 2.5×10^{-5} M Ce(IV); 0.5 M H_2SO_4.

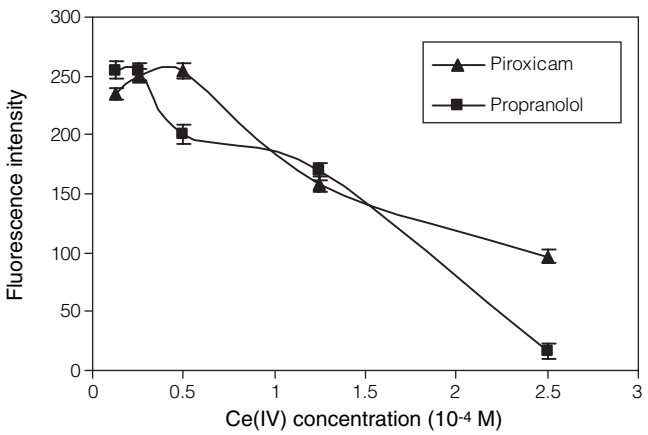

Figure 3. Effect of Ce(IV) concentration on the spectrofluorimetric responses: 0.5 mg/L piroxicam or propranolol; 1.0 M H_2SO_4.

to insufficient Ce(IV) for oxidation. On the other hand, higher concentrations of Ce(IV) was reported to probably quench the fluorescence thus decreasing the detected intensity[32,33,35]. An aliquot of 0.2 or 0.1 mL of Ce(IV) (final concentration of 5.0 or 2.5×10^{-5} M) was used for the oxidation of piroxicam or propranolol, respectively.

II. *Effect of H_2SO_4 Concentration*

The effect of H_2SO_4 concentration on the fluorescence intensity was depicted in Figure 4. It was observed that the fluorescence intensity remained approximately constant at the studied concentration range. Hence, an aliquot of 1.0 mL H_2SO_4 (final concentration of 0.5 M) was taken as optimum for other experiments.

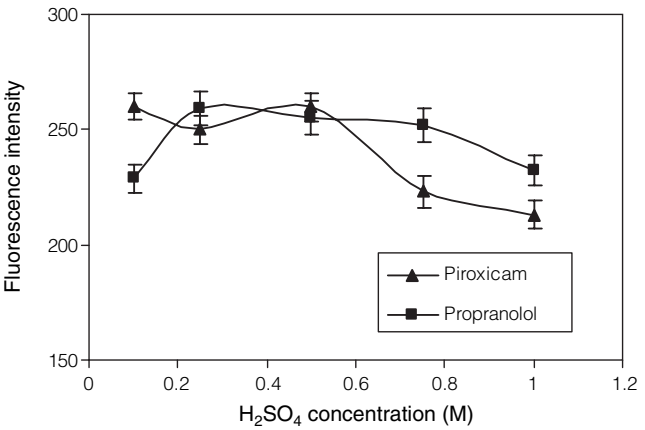

Figure 4. Effect of H_2SO_4 concentration on the spectrofluorimetric responses: 0.5 mg/L piroxicam or propranolol; 5.0×10^{-5} or 2.5×10^{-5} M Ce(IV) in the case of piroxicam or propranolol, respectively.

Table 1. Analytical characteristics of the methods

Analyte	Method (Spectrofluorimetry)	Concentration range (mg/L)	Slope	Intercept	r^2	RSD (%)	LOD (mg/L)	Ref.
Piroxicam	Monitoring of Ce(III) fluorescence	0.02 – 3.0	276.8	-13.32	0.9976	1.65	0.006[a]	This work
	Direct spectrofluorimetry	0.01–1.25	16.3	1.0	0.998 (r)	1.20	0.012	1
	Indirect spectrofluorimetry	0.2 – 8.0	–	–	–	–	–	13
	Europium sensitized fluorescence	100 – 2000 ppb	–	–	–	2 and 3	23.0 ppb	14
	Solid-phase extraction and room-temperature fluorimetry	0.03 – 0.2	42.3	1.02	0.993 (r)	–	0.010	15
	Micelle-enhanced fluorescence	0.05 – 1.5	–	–	–	–	0.015	16
	Spectrofluorimetry in the presence of β-cyclodextrin	0.02 – 1.0	28.6	2.9	0.999 (r)	–	0.02	17
Propranolol	Monitoring of Ce(III) fluorescence	0.02 – 2.4	269.9	-3.44	0.9969	1.79	0.008[a]	This work
	Indirect spectrofluorimetry	0.4 – 18.0	–	–	–	–	–	13
	Synchronous spectrofluorimetry	6 – 200 ppb	1.446×10^{-3}	3.07×10^{-4}	0.9997	1.50	1.9 ppb	18
	Fluorimetry (sequential injection analysis)	0.0 – 4.0	4246.8	−44.9	> 0.999	2.35	0.02	19
	Synchronous spectrofluorimetry	0.02 – 1.0	–	–	–	–	–	20

[a]Determined as three times the standard deviation of the blank signals.

Table 2. Tolerance limits of interfering species in the determination of 1.0 mg/L of piroxicam or propranolol

Additive	Tolerance concentration ratio (C/C)	%Recovery (n = 3) In the case of piroxicam	%Recovery (n = 3) In the case of propranolol
Magnesium stearate	120	101.2 ± 1.62	99.6 ± 1.72
Sucrose	100	98.7 ± 1.58	100.5 ± 1.81
Lactose	60	99.5 ± 1.64	98.4 ± 1.74
Glucose	50	98.8 ± 1.57	99.5 ± 1.83
Saccharose	50	98.5 ± 1.54	98.1 ± 1.76
Citric acid	10	97.6 ± 1.57	98.7 ± 1.77
Propylene glycol	2	102.3 ± 1.70	101.7 ± 1.79

Table 3. Determination of piroxicam and propranolol in pharmaceutical preparations

Method	Piroxicam content*		Tabulated t and F values**
	Proposed method	Direct spectrofluorimetric method	
Piroxicam capsule (10 mg/capsule)	10.4 ± 0.17	10.2 ± 0.14	t = 1.57, F = 1.47
Piroxicam gel (0.5 g/100g)	0.5 ± 0.01	–	
Propranolol tablet (10 mg/tablet)	10.3 ± 0.18	10.1 ± 0.15	t = 1.48, F = 1.44

*Average of three determinations ± standard deviation.
**Tabulated t and F values at p = 0.05 and n = 4 are 19 and 2.78, respectively[36].

III. *Effect of Temperature and Time*

The proposed methods involve the rapid reaction between piroxicam or propranolol and Ce(IV) at ambient temperature without heating. Whereas, heating at high temperatures for several minutes has been reported in other methods for the determination of macrolide antibiotics and psychoactive drugs[32,33]. The effect of equilibration time on fluorescence intensity was also investigated and the results indicated that an equilibration time of 10 min is adequate to obtain the maximum fluorescence intensity.

IV. *Characteristics of the Method*

The linear concentration range, relative standard deviation (RSD) and the limit of detection (LOD), calculated as three times the standard deviation of the blank signals, for piroxicam and propranolol are shown in Table 1. The obtained LOD for piroxicam and propranolol was 0.006 and 0.008 mg/L, respectively. The RSD for 5 replicate determinations of piroxicam and propranolol at 1.0 mg/L concentration level was 1.65 and 1.79%, respectively. The figures of merit of our methods, which were compared with other spectrofluorimetric methods as shown in Table 1, are comparable or superior among these methods.

V. *Interference Study*

In order to evaluate the possible analytical applications of the proposed method, the influence of frequently encountered excipients and additives were studied by analyzing sample solutions containing 1.0 mg/L of piroxicam or propranolol with different amounts of possible interferents. The tolerance limit was taken as the concentration causing an error less than 5% in the determination of the drug as shown in Table 2. Besides, the obtained recovery indicated that no serious interference occurred from the classical additives tested.

VI. *The Validation and Application of the Method*

The proposed method was successfully applied to the analysis of piroxicam or propranolol in commercial pharmaceutical preparations (Table 3). Statistical

Table 4. Results of recoveries of commercial dosage form

Dosage form	Prepared solution	Piroxicam or propranolol added (mg/L)	Piroxicam or propranolol found (mg/L)*	C.V%	E%	Recovery (%)
Piroxicam capsule	0.5 mg/L					
		0.2	0.69 ± 0.01	1.45	-5	95
		0.5	0.99 ± 0.02	2.02	-2	98
		1.0	1.46 ± 0.02	1.37	-4	96
Piroxicam gel	0.5 mg/L					
		0.2	0.71 ± 0.01	1.41	+5	105
		0.5	1.01 ± 0.02	1.98	+2	102
		1.0	1.58 ± 0.03	1.89	+8	108
Propranolol tablet	0.5 mg/L					
		0.2	0.70 ± 0.01	1.43	0	100
		0.5	1.04 ± 0.02	1.92	+8	108
		1.0	1.53 ± 0.03	1.96	+3	103

*Average of three determinations ± standard deviation.

analysis of the obtained results was carried out at 95% confidence interval and no significant difference was observed with regard to the accuracy and precision. The piroxicam or propranolol content measured by the proposed methods was in excellent agreement with those obtained by direct spectrofluorimetric methods[1,19].

The validity of this method was further assessed by spike – recovery test on solutions prepared from piroxicam or propranolol formulations (Table 4) with recoveries ranging from 95% to 108%. The recovery, along with the coincidence of excitation and emission spectra of drug formulations to those of the standard solution of piroxicam or propranolol (see Figure 1 or 2), indicated that no significant matrix effect was observed in the proposed procedure.

CONCLUSIONS

This report describes a validated spectrofluorimetric method for the assay of piroxicam and propranolol without interference of common excipients. This is recommended as a method for piroxicam and propranolol testing either in bulk or the corresponding dosage forms in routine quality control. The sensitivity is comparable to the existing spectrofluorimetric methods for the determination of piroxicam and propranolol with wider linear dynamic range (LDR) in most cases. In addition, the LOD and LDR of this method are comparable or better than those of other methods using Ce(IV) as oxidant for pharmaceutical compounds[32-34]. From the economic point of view, the proposed method is simple, rapid and inexpensive, and thus seems a good alternative to previously reported methods.

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