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A sensitive fluorescence optosensor for analysing propranolol in pharmaceutical preparations and a test for its control in urine in sport

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Abstract

We describe a simple and selective method for analysing propranolol and a sensitive test for its control in urine. A flow-through fluorescence optosensor based on on-line immobilization in a non-ionic-exchanger (Amberlite XAD-7) solid support in a continuous flow was used in both cases. Determination was made in 5 mM $H_2PO_4^-/HPO_4^{2-}$ buffer solution at pH 6 at a working temperature of 20 °C. Fluorescence intensities were measured at $\lambda_{ex/em} = 300/338$ nm with a response time of 80 s, thus obtaining a linear concentration range of between 0 and 250.0 ng ml^{-1} with a detection limit of 1.3 ng ml^{-1}, an analytical sensitivity of 6.0 ng ml^{-1} and a standard deviation of 2.40% at a 150 ng ml^{-1} concentration level for propranolol. We also propose a test to detect propranolol in urine with a linear concentration range between 0 and 100.0 ng ml^{-1}, a detection limit of 0.2 ng ml^{-1}, an analytical sensitivity of 1.0 ng ml^{-1}, and a standard deviation of 0.84% at a 75 ng ml^{-1} concentration level. The effect of proteins presents in urine samples were evaluated. The two proposed methods were satisfactorily applied to commercial formulations and urine samples respectively.

Keywords: Flow-through fluorescence sensor; Propranolol; Pharmaceutical analysis; Urine; Doping test

1. Introduction

Propranolol is a beta-adrenergic blocking drug widely prescribed for the treatment of cardiac arrhythmia, sinus tachycardia, angina pectoris and hypertension [1,2]. It has also been suggested for use in a number of other conditions including dysfunctional labour and anxiety. When administered over a long period of time it reduces mortality caused by hypertension and lengthens survival in patients with coronary heart disease [3,4].

It is also used in low activity sports, reducing cardiac frequency, contraction force and coronary flow [5]. Therefore, it has been included in the list of forbidden substances by the International

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Olympic Committee [6]. The Spanish Olympic Committee has decided that only a qualitative determination of propranolol in urine is necessary [7].

Different techniques [8–13], including fluorimetry [5–14] and phosphorimetry [15], have hitherto been used to determine propranolol in commercial formulations and/or biological fluids. Practically all previous methods function in a batch system and require various tedious preliminary procedures such as pre-concentration in an organic solvent. Thus, in recent years new techniques to determine propranolol such as molecularly imprinted [16] or ion selective PVC membrane electrodes [17] have been developed, but it is still necessary to further develop highly selective, simple, rapid and cheap procedures to determine propranolol in pharmaceutical preparations and human fluids.

The analytical advantages of optical sensors have been demonstrated during the last decade [18,19]. A combination of flow-injection analysis techniques with detection on optically active surfaces with an immobilised indicator packed in a flow-through cell, has been called 'optosensing flow-injection analysis' [20] and has proved to offer important advantages because of its high sensitivity, selectivity, precision, simplicity, speed and low cost [21].

The development of optosensing techniques has led to a shorter turnaround analysis time and reduces costs for doping controls. As a large part of the samples prove to be non-doped, rapid analytical methods such as doping tests that provide reliable 'yes/no' responses are of increasing interest. These tests can usually be described as systems that 'filter' samples to select those with analyte content levels 'similar to' or 'higher than' a previously established threshold. These 'probably doped' samples must then be examined with more exact instrumental methods. Doping tests can significantly cut costs and save time [22,23].

We have developed an optosensor for the drug propranolol to use in the analysis of pharmaceutical preparations and as a doping test for the qualitative analysis of propranolol in human urine without lengthy preliminary procedures.

2. Experimental

2.1. Chemicals and materials

Analytical reagent grade chemicals were used for the preparation of all the solutions. Sodium dihydrogen phosphate 1-hydrate was bought from Sigma (Spain) and used as received. A solution of 0.1 M $H_2PO_4^-/HPO_4^{2-}$ buffer at pH 6.0 was freshly prepared.

A solution of propranolol (25 μg ml^{-1}) (Sigma) was prepared in distilled water.

The strong basic anion-exchanger resins Dowex 1x2-100, Dowex 1x4-100 and Dowex 1x8-100 (Sigma), the strong acid cation-exchanger resins Dowex 50wx2-100, Dowex 50wx4-100 and Dowex 50wx8-100 (Fluka), and the non-ionic resins Amberlite XAD 2, Amberlite XAD 4, Amberlite XAD 7, Silica Gel Davisil and Silica Gel Merck (Sigma) were sieved and then used at three-grain size (80–120, 120–160 and >160 μm).

Water was distilled twice and prepared with a Mili-Q System (Millipore, Bedford, MA).

2.2. Optosensing manifold

Using a single-line flow-injection system, a Hellma Model 176.052-QS flow-through cell of 25 μl volume was packed with the corresponding resin and placed in the standard sample compartment of the detector. Two rotatory valves (Supelco 5020) were used for sample introduction and renewing the active surface. PTFE tubing (0.8 mm i.d.) and fittings were used for connecting the flow-through cell. A Gilson Miniplus-3 peristaltic pump was used to generate the flow stream.

All fluorescence measurements (relative fluorescence intensity, R.F.I.) were carried out with an Aminco Bowman Series 2 luminescence spectrometer equipped with a continuous high-power xenon lamp and a thermostat cell holder.

2.3. General procedure

A 2-ml of the sample was injected through valve of sample into a channel of 5 mM $H_2PO_4^-/HPO_4^{2-}$ buffer solution, pH 6, into the carrier stream. In this medium at a 1.5 ml min^{-1} flow-rate the

propranolol is retained in the flow cell on Amberlite XAD 7. After the fluorescent measurement ($\lambda_{exc/em} = 300/338$ nm, detector voltage of 600 V, slits of 4 nm for excitation and emission) 250 μL of regenerative solution (acetonitrile/H_2O 80% v/v) was injected through valve of regenerative solution to strip the analyte retained on the solid phase before proceeding with the next sample.

The measurement was repeated three times to evaluate its error.

2.4. Procedure for pharmaceutical preparations

Sumial 10 mg (Laboratorios Seneca), a commercial product bought at the local chemists, with a nominal content of 10 mg of propranolol and an unknown concentration of excipients, was analysed. The contents of five pills were powdered and homogenised. A portion was dissolved in doubly distilled water and an aliquot of this solution was treated as indicated under general procedure.

2.5. Procedure for urine samples

Doped urine was collected 24 h after the oral intake of a 5 mg single dose (half a pill) of Sumial 10 mg. Propranolol-free urine was also collected from two volunteers (male and female).

Both types of samples were vortexed 3800 r.p.m. for 15 min and frozen until preparation for analysis. For the analysis of the urine samples, aliquots of these were treated as indicated under general procedure.

3. Results and discussion

3.1. Selection of reagent phase and regenerative solution

The strong basic anion-exchanger resins Dowex 1x2-100, Dowex 1x4-100 and Dowex 1x8-100, the strong acid cation-exchanger resins Dowex 50wx2-100, Dowex 50Wx4-100 and Dowex 50Wx8-100 and the non-ionic resins Amberlite XAD 2, Amberlite XAD 4, Amberlite XAD 7, Silica Gel Davisil and Silica Gel Merck were studied (see Table 1). The best differences between noise (due to resin signal) and the fluorescence signal of propranolol were obtained using Amberlite XAD 7. Using this resin, different grain sizes were tested and improvements in the analytical signal were observed with 80–120 μm mesh resin.

Table 1
Selection of reagent phase

Support	I^a
Dowex 1x2-200	0.031
Dowex 1x4-200	0.056
Dowex 1x8-200	0.017
Dowex 50wx2-200	0.378
Dowex 50wx4-200	0.136
Dowex 50wx8-200	0.068
Silica Gel Davisil	0.093
Silica Gel Merck	0.350
Amberlite XAD 2	0.104
Amberlite XAD 4	0.070
Amberlite XAD 7	1.580

[a] Differences between fluorescence and noise signals (2 ml of propranolol 150 ng ml^{-1} were injected in all cases).

Finally, we made experiments to find the most suitable regenerative solution to make the system satisfactorily reusable. This proved to be acetonitrile/water 80% v/v.

3.2. Fluorescence properties on solid surface

The fluorescence excitation and emission spectra of propranolol on the non-ionic resin were recorded and shown in Fig. 1. Propranolol on Amberlite XAD 7 emits fluorescence with a

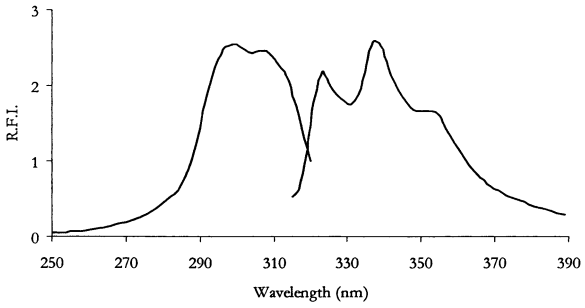

Fig. 1. Excitation and emission spectra of propranolol. [Propranolol] = 150 ng ml^{-1}, 5×10^{-3} M $H_2PO_4^-/HPO_4^{2-}$ buffer solution at pH 6, voltage detector 600 V and $slits_{exc/em}$ 4/4 nm.

maximum excitation intensity at 300 nm and maximum emission intensity at 338 nm. Different instrumental parameters relating to the luminescence technique affect fluorescence intensity and so these were carefully optimised (see Table 2).

3.3. Influence of pH, type and concentration of buffer solution

pH affects the dissociation of the compound and consequently the retention on the exchanger resin. The effect of pH on the fluorescence emission intensity of propranolol and noise signals are shown in Fig. 2.

Different pH values ranging from 2.0 to 13.0 were studied under optimum conditions, fixing the quantity of propranolol at 150 ng ml^{-1}. Under these conditions, propranolol presents maximum fluorescence at pH 6.0.

Different buffer solutions (phosphate/HCl and ftalate/NaOH) were tested at pH 6. The best results were obtained with phosphate/HCl. Different concentrations of phosphate/HCl buffer solution (between 0 and 20×10^{-3} M) were also tested, the optimum concentration proving to be 5×10^{-3} M.

3.4. Optimisation of FIA variables

The retention of propranolol changes according to the carrier flow-rate. An increase in flow-rate significantly decreases the fluorescence signal but also decreases the response time of the optosensor (time passed between injection and maximum intensity measurement). Thus, an optimum value of 1.5 ml min^{-1} was chosen for the rest of our experimental work (see Fig. 3a)

Table 2
Instrumental parameters

	Optima values
Wavelength excitation/emission	300/338 nm
Detector voltage	600 V
Slits (excitation/emission)	4/4 nm
Resolution	5 s

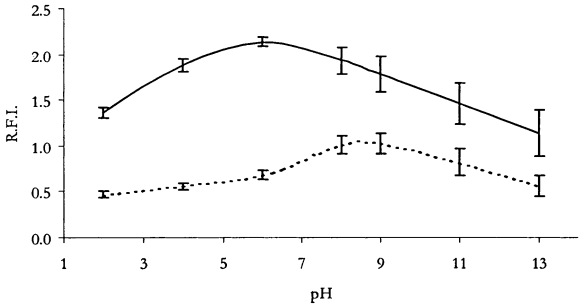

Fig. 2. Effect of pH on the fluorescence (—) and noise (---) signals. [Popranolol] = 150 ng ml^{-1}, $\lambda_{exc/em}$ = 300/338 nm, voltage detector 600 V, $slits_{exc/em}$ 4/4 nm, resolution 5 s, flow-rate 1.5 ml min^{-1} and injection volume 2 ml.

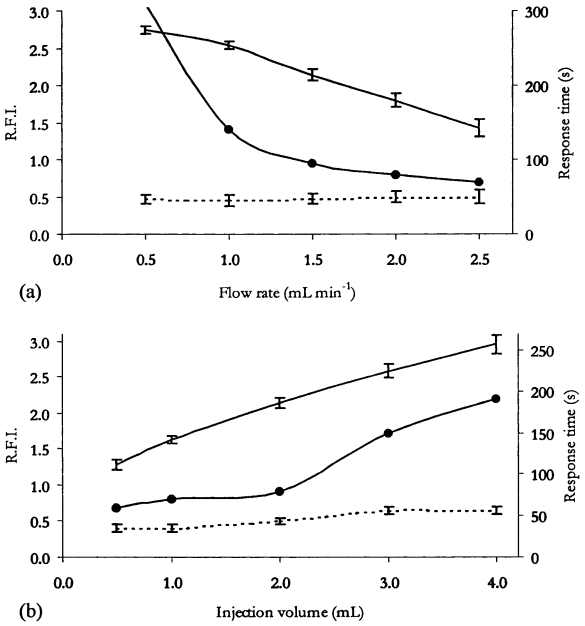

Fig. 3. Effect of flow-rate (a) and injection volume (b) on the fluorescence of propranolol (—), noise (---) and response time (-●-●-) signals. [Propranolol] = 150 ng ml^{-1}. Experimental and instrumental parameters as Figs. 1 and 2, respectively.

The injection volume of propranolol considerably affects the fluorescence emission signals. An increase in injection volume of up to 3.0 ml increases the fluorescence signal intensity concomitantly but an increase in injection volume also increases the sensor time response. Therefore, an injection volume of 2.0 ml was chosen as the optimum value (see Fig. 3b).

3.5. Effect of temperature

Temperature is normally an important parameter affecting fluorescence emission intensity and the analyte retention on the exchanger resin. A detailed study of the effect of temperature upon the fluorescence emission of propranolol was carried out at temperatures ranging from 5 to 50 °C. The fluorescence signal was virtually unchanged within this range so it was unnecessary to fix any particular temperature.

3.6. Calibrations, sensitivity and precision

To evaluate the performance of our analytical methodology standard linear calibration graphs were drawn according to recommended procedures, with the curve passing through the origin for propranolol.

The regression equation was $I = 0.072 + 0.009C$, where C is the concentration in ng ml^{-1} of propranolol and I is the difference between the analyte and noise signals. The correlation coefficient (r) was 0.998.

The wide linear range, small standard errors and correlation coefficient indicate very good calibration linearity. The limit of detection was determined using the IUPAC method ($\mathrm{LOD} = 3S_b/m$) and quantification limit, sensitivity and precision were determined using the method proposed by Cuadros et al. [24]. All the features of the proposed method are summarised in Table 3.

Table 3
Analytical parameters of proposed method

	Estimate value
Relative standard deviation (R.S.D. (a)) (%)	32.35
Relative standard deviation (R.S.D. (b)) (%)	1.65
Standard deviation of regression ($s_{R,c}$)	0.055
Linearity (%)	98.35
Linear range (ng ml^{-1})	0–250
Sensitivity (ng ml^{-1})	6.0
Limit of detection (ng ml^{-1})	1.3
Limit of quantification (ng ml^{-1})	42.6
Precision (R.S.D.) (%)	
50 ng ml^{-1}	7.6
100 ng ml^{-1}	4.0
150 ng ml^{-1}	2.4
200 ng ml^{-1}	1.9
250 ng ml^{-1}	1.7

3.7. Analysis of propranolol in pharmaceutical preparations

The detection of low levels and good analytical sensitivity of the proposed method facilitate the determination of propranolol in pharmaceutical preparations.

Our proposed method was applied to the determination of propranolol in a pharmaceutical preparation called Sumial 10 mg, without matrix effect (because of the similarity of fluorescence intensity and the coincidence of excitation and emission spectra between the pure analyte and the pharmaceutical preparations) in which we obtained a percentage recovery of 98.74% with a relative deviation standard of 1.46% for seven replicas.

3.8. Doping test for propranolol

Propranolol is a beta-adrenoceptor that is used in sports demanding low physical activity. Propranolol is rapidly and almost completely absorbed after oral administration and undergoes extensive first-pass metabolism. During 48 h less than 4% of a dose is excreted in the urine as an unchanged drug [25].

The Spanish Olympic Committee has decided that only a qualitative determination of propranolol in urine is necessary [7]. The minimum detectable quantity of propranolol in urine is the detection limit of the proposed method in the presence of urine samples.

The performance of the proposed analytical test was evaluated by establishing a standard linear calibration graph according to recommended procedures adding free-propranolol urine samples (until 1:10 dilution) to evaluate and eliminate the matrix effect of the urine in the proposed test.

The regression equation was $I = -0.179 + 0.005C$, where C is the concentration in ng ml^{-1} of propranolol and I is the difference between the analyte signal and the noise signal. The correlation coefficient (r) was 0.999. The calibration graph has a negative origin because there is a quenching

effect on the fluorescence intensity from the urine matrix, although a linear increase of I with propranolol concentration was observed.

The wide linear range, small standard errors and correlation coefficient indicate very good calibration linearity. The detection limit was determined using the IUPAC method and quantification limit, sensitivity and precision were determined using the method proposed by Cuadros et al. [24]. All the features of the proposed method are summarised in Table 4.

The Amberlite XAD 7 is commonly used for protein purification. In addition, the proportions of individual proteins excreted in the urine depend on the extent of their reabsorption by the renal tubules; albumin represents approximately 60% of total proteins excreted because it is not completely removed from the filtrate by the tubular cells [26]. The values of albumin in urine can fluctuate between 0 and 80 mg l^{-1}. To evaluate the effect of proteins in the determination of propranolol using the doping test, three different levels (80, 160 and 800 mg l^{-1} of albumin) were added to the urine spiked sample observing that the very-high level of proteins (albumin) do not affect the response of the doping test proposed and there is not a gradual accumulation of proteins on the solid support because the proteins are removed with the regenerative solution.

To demonstrate that the doping test is a good tool for controlling propranolol in urine, three urine samples (one doped, from a male volunteer, and two propranolol-free urine samples, from a male and female volunteers) were checked (see Fig. 4).

We have shown that there is a difference between free-propranolol urine samples and doped urine sample signals and demonstrate that the doping test is able to detect the ingestion of only 5 mg of propranolol 24 h after the volunteer has taken the medicine.

Table 4
Analytical parameters of proposed doping test

	Estimate value
Relative standard deviation (R.S.D. (a)) (%)	1.2
Relative standard deviation (R.S.D. (b)) (%)	0.7
Standard deviation of regression ($s_{R,c}$)	0.005
Linearity (%)	99.3
Linear range (ng ml^{-1})	0–100
Sensitivity (ng ml^{-1})	0.96
Limit of detection (ng ml^{-1})	0.2
Limit of quantification (ng ml^{-1})	7.0
Precision (R.S.D.) (%)	
25 ng ml^{-1}	2.5
50 ng ml^{-1}	1.2
75 ng ml^{-1}	0.8
100 ng ml^{-1}	0.7

4. Conclusions

We present an optosensor for the on-line determination of propranolol in pharmaceutical preparations and a doping test for propranolol in urine. The proposed method offers excellent analytical parameters, such as sensitivity, selectivity, versatility, and ease of use. These may well be a good alternative to more sophisticated techniques for the analysis of biological samples and for controlling the concentrations of pharmaceutical preparations.

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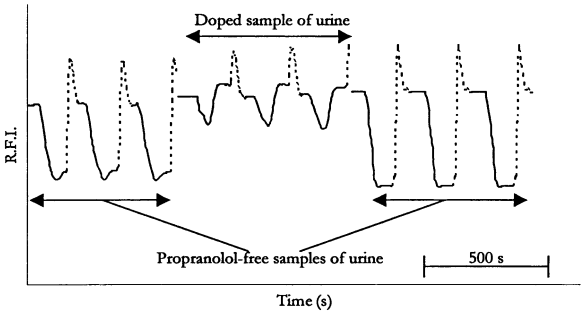

Fig. 4. Signal obtained using the test for propranolol in two propranolol-free samples of urine and a doped sample of urine. (—) Signal when the carrier is passing through the active surface, (---) signal when the regenerative solution is passing through the active surface.

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