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Facile and selective determination of the cerebral vasodilator nafronyl in a commercial formulation by heavy atom induced room temperature phosphorimetry

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Abstract

This paper presents a facile and selective method for the determination of the pharmaceutical compound nafronyl using heavy atom induced room temperature phosphorimetry (HAI-RTP) as analytical technique. The determination was performed in potassium iodide 1.6 M and sodium sulphite 0.002 M at a measurement temperature of 20°C. The phosphorescence intensity was then measured at $\lambda_{exc} = 292$ nm and $\lambda_{em} = 524$ nm. Phosphorescence was fully developed instantly, obtaining a linear concentration range between 2.7 and 250 ng ml^{-1} with the detection limit of 2.7 ng ml^{-1}, an analytical sensitivity of 5.1 ng ml^{-1} and a standard deviation of 2.17% at a 150 ng ml^{-1} concentration level. The proposed method has been satisfactorily applied to the unique Spanish commercial formulation containing nafronyl at a 100 mg level per capsule. The recovery was 108% with a 1.7% standard deviation of the analytical measurement. The method has been validated using standard addition methodology.

Keywords: Pharmaceutical analysis; Nafronyl; Heavy atom induced (HAI); Room temperature phosphorescence (RTP)

1. Introduction

Nafronyl (NFL) is a vasodilator used for the treatment of cerebral and peripheral vascular disorder [1–3]. Unfortunately, this vasodilator agent can be consumed as a doping substance in order to prevent cerebral and peripheral circulation insufficiency, as well as arteriopathies of lower members (cramps and spasms), altering the outcomes of sports competition. The uncontrolled use of this drug could cause serious health endangering secondary effects. For this reason, since 1990 the International Olympics Committee, has included it in its list of forbidden substances [4].

Few reports on the determination of nafronyl at therapeutic levels have been published [5–11]. Previous efforts used mainly liquid chromatography which require various tedious preliminary

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procedures such as preconcentration in an organic solvent. For this reason, it is necessary to develop highly selective and facile procedures for the determination of nafronyl in pharmaceutical preparations.

Phosphorescence methodologies [12,13] offer the advantages of wide linear dynamic ranges, low detection limits and great selectivity. Micelle-stabilised RTP [10,11] combined with sodium sulphite as an oxygen scavenger have permitted the determination of nafronyl in different matrices in solution.

Our research group has proposed a simplification, not based the use of organised media. In previous studies [14], it has been demonstrated that the RTP emission of naphthalene derivatives can be directly induced in aqueous solutions only by the addition of high concentrations of a heavy atom perturber and sodium sulphite as a chemical deoxygenator. This new methodology has been named Heavy Atom Induced-RTP (HAI-RTP) [14]. The first analytical applications of this methodology were carried out by our research group on two naphthalene derivatives, the plant growth regulator β-naphthoxyacetic acid [15] and the drug naphazoline in pharmaceutical preparations [16]. Li and co-workers [17–19] also corroborated these findings declaring that this phosphorescence emission is not a specific phenomenon of certain derivatives but a regular characteristic of naphthalene derivatives.

This paper presents a new phosphorimetric methodology for the determination of nafronyl in fluid solution by a simple procedure that has demonstrated its applicability in the unique Spanish commercial formulation containing 100 mg of the analyte per capsule and it could also be used as a procedure for routine control in the International Olympic Committee regulation of forbidden substances.

2. Experimental

2.1. Instrumentation

All recordings of uncorrected luminescence spectra and measurements of HAI-RTP intensities were carried out with an Aminco Bowman Series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp and a thermostatted cell holder. The system was controlled with a personal computer with 4 MB RAM memory, OS/2 version 2.0, and a GPIB (IEEE-488) interface card for computer-instrument communication.

2.2. Reagent and solutions

Analytical reagent-grade chemicals were employed for the preparation of all the solutions. Thallium (I) nitrate, potassium iodide, potassium bromide, potassium chloride, sodium iodide, sodium bromide, sodium chloride, rubidium chloride, cesium chloride and anhydrous sodium sulphite were purchased from Sigma Chemical Co. (Spain) and were used as received. Solutions with a concentration of 2 M were prepared for most heavy atom salts. Thallium nitrate solution at a concentration 0.25 M was prepared. Freshly prepared sodium sulphite was present in all solutions at a concentration of 0.1 M.

Aqueous solutions (50 μg ml^{-1}) were prepared for NFL (Sigma Chemical Co.)

The water used was distilled twice and prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. General procedure

A 20 μl aliquot of NFL stock solution with 8 ml of 2 M potassium iodide and 200 μl of 0.1 M sodium sulphite were introduced into a 10 ml calibrated flask and made up to volume with water. Standard 10 mm fused silica cells were used.

The phosphorescence intensities of the samples and the corresponding blanks were measured at the phosphorescence wavelength maxima of $\lambda_{exc}/\lambda_{em}$ 292/524 nm, $slits_{exc/em}$ 16/16 nm, $t_{d/g}$ (decay time and gate time) 150/100 μs and voltage detector 1100 V.

2.4. Sample preparation

The proposed method has been applied to the analysis of NFL in the unique commercial formu-

lation, purchased in Spain, called Praxilene 100 mg (Productos Farmacéuticos FAES, Bilbao, Spain).

The nominal content per capsule is: 100 mg of nafronyl accompanied by talc, microcrystalline silica, magnesium stearate and other excipients without indications of their concentration.

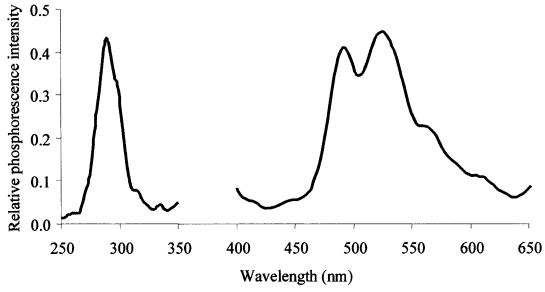

Fig. 1. Projected two-dimensional spectrum of NFL. [NFL] = 100 ng ml^{-1}, [KI] = 1.6 M, [Na_2SO_3] = 0.002 M. Emission, 400–650 nm, Excitation, 250–350 nm, $slits_{exc/em}$, 16/16 nm, $t_{d/g}$, 150/100 μs, and voltage detector, 1100 V.

Table 1
Instrumental parameters

Instrumental parameters	Optimum values
Wavelength excitation/emission	292/524 nm
Delay time (t_d)	150 μs
Gate time (t_g)	100 μs
Slits (excitation/emission)	16/16 nm
Detector voltage	1100 V
Scan speed	2 nm s^{-1}
Minimum period of pulse	5 ms

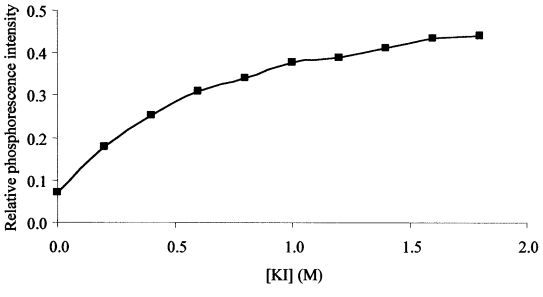

Fig. 2. Influence of KI concentration on the HAI-RTP of NFL. [NFL] = 100 ng ml^{-1}, [Na_2SO_3] = 0.002 M. $\lambda_{exc}/\lambda_{em}$ 292/524 nm, $slits_{exc/em}$, 16/16 nm, $t_{d/g}$, 150/100 μs, and voltage detector, 1100 V.

The contents of five capsules were mixed and taken for analysis and the solid was powdered and homogenised. A portion was dissolved in doubly distilled water and several aliquots of this solution were treated as indicated under general procedure.

3. Results and discussion

3.1. Phosphorescence properties

Fig. 1 shows the excitation and phosphorescence emission spectra of nafronyl in aqueous solution. NFL emits phosphorescence with a maximum excitation intensity at 292 nm and two maximum emission intensities at 492 and 524 nm. The best signal/noise relation was obtained at the wavelength emission of 524 nm. The triplet lifetime of nafronyl was determined as 300 μs.

Different instrumentals parameters related to the luminescence technique could also affect the phosphorescence response, so they should be carefully selected (see Table 1). All these optimal instrumental parameters were kept constant for the rest of the experimental work.

3.2. Influence of heavy atom perturbers

Different heavy atoms salts (KI, NaI, KBr, NaBr, KCl, NaCl, RbCl, CsCl, $TlNO_3$) were studied as heavy atom perturbers to observe the HAI-RTP emission of the NFL. A phosphorescence signal was observed only, by using KI. No-phosphorescence response of NFL is obtained in the total absence of KI while more intense HAI-RTP intensity was observed as the heavy atom concentration increased.

As a result of these studies, the optimum concentration of KI selected for the observation of phosphorescence emission of NFL was 1.6 M to obtain the maximun response (see Fig. 2).

3.3. Influence of deoxygenation and pH

There are different methods described in the literature to carry out the deoxygenation process needed to observe phosphorescence signals in so-

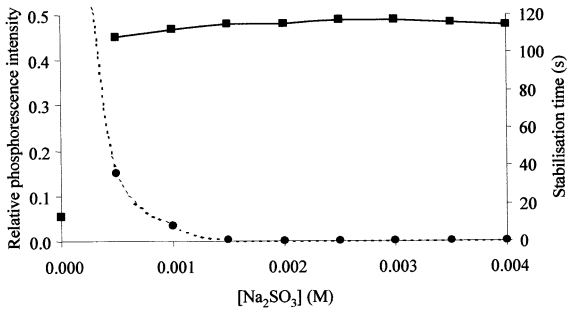

Fig. 3. Influence of sodium sulphite concentration on the HAI-RTP of NFL. [NFL] = 100 ng/ml, [KI] = 1.6 M. The instrumental parameters as Fig. 2.

lution. These consist mainly of treatment with inert gas [20] and sodium sulfite [21]. Besides classical methods, we have introduced two new methods of deoxygenation for HAI-RTP using $Zn_{(s)}$/HCl or Na_2CO_3/HCl to produce inert gases such as $H_{2(g)}$ or $CO_{2(g)}$ in the reacting media [22]. The $H_{2(g)}$ or $CO_{2(g)}$ generated remove the dissolved oxygen, but a phosphorescence signal was not observed.

In this work, sodium sulphite was selected as deoxygenation scavenger. In aqueous, solution the elimination of dissolved oxygen is practically immediate. The study was performed by monitoring the signal as a function of time until the HAI-RTP signal was stabilised for at least 2 min. To find the optimum concentration of sodium sulphite, different amounts between 0.5 and 4.0 mM were added to a solution with a fixed concentration of the analyte under the study in presence of the heavy atom salt selected. A 2 mM sodium sulphite solution was selected as optimal (see Fig. 3).

Various pHs, from 3.6 to 10.2, were tested with a solution with a fixed amount of NFL, heavy atom salt and sulphite. With pHs greater than 5.6 the HAI-RTP signal was constant and the stabilisation time was practically instantaneous.

In these optimised experimental conditions the system had a pH of 8.12, so the use of a buffer solution was not needed. Accordingly, it was not used for the rest of the experimental work.

3.4. Effect of organic solvents on HAI-RTP intensities

The effects of different percentages of five organic solvents miscible with water were studied in order to improve phosphorescent measurement and facilitate the solubilization of NFL in aqueous solution.

The study carried out demonstrated no change in Relative Phosphorescence Intensity (RPI) with the different solvents but an increase in stabilisation time was observed. When methanol was tested, good RPI and stabilisation time were obtained up to 10% of the co-solvent present. This percentage could be chosen in order to increase solubilisation of NFL in water solutions.

3.5. Effect of temperature and stability

We have carried out a detailed study of the effect of temperature on the HAI-RTP emission in the range 5–40°C. This study confirms that the RTP intensity decreases almost linearly as the temperature is increased. A temperature of 20°C, was chosen to facilitate the experimental work.

Under these experimental conditions, RTP signals for the NFL are obtained instantaneously and remain stable for, at least, 1 h.

3.6. Calibrations, sensitivity and precision

Analytical performance characteristics of the proposed methodology were evaluated. Standard calibration graph, prepared according to recommended procedures, were linear passing through the origin for the NFL. The regression equation was

$$\mathrm{RPI} = 0.091 + 0.007 \cdot C,$$

where C is the concentration of NFL in ng ml^{-1}. The correlation coefficient $(r) = 0.9984$ $(n = 7)$.

The wide linear range, small standard errors and correlation coefficient indicate very good calibration linearity. The detection limit and quantification limit have been calculated according to IUPAC [23] and sensitivity and precision, expressed as relative standard deviation, was determined using the method proposed by Cuadros et.

al. [24]. All the features of the proposed method are summarised in Table 2.

3.7. Analytical applications

The low detection limits and the good analytical sensitivity of the proposed method makes possible the determination of nafronyl in pharmaceutical preparations.

The proposed method was applied to the determination of nafronyl in the unique Spanish pharmaceutical preparation that contain this vasodilator agent called *Praxilene* 100 mg.

The samples were analysed using the phosphorimetric method described above. Fig. 4 shows the similarity between nafronyl and Praxilene emission spectra under the same instrumental and experimental conditions. This similarity indicates that no eventual interferent effect of the formulation excipients exist.

Also, the method was validated with a standard addition method of calibration. The concentration obtained was 108 mg/capsule.

Table 2
Analytical parameters of proposed method

Lineal range (ng ml^{-1})	2.7–250
Sensitivity (ng ml^{-1})	5.1
Detection limit (ng ml^{-1})	2.7
Quantification limit (ng ml^{-1})	8.1
RSD (%) (150 ng ml^{-1})	2.17

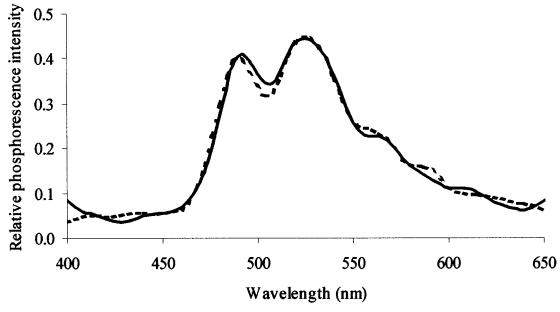

Fig. 4. Comparison between emission spectrum of NFL, (—) and Praxilene (---). [NFL] = 100 ng ml^{-1}, [Praxilene] = 100 ng ml^{-1}, [KI] = 1.6 M, [Na_2SO_3] = 0.002 mM. Emission, 400–650 nm, λ_{exc}, 292 nm, $slits_{exc/em}$, 16/16 nm, $t_{d/g}$, 150/100 μs, and voltage detector, 1100 V.

To check the similarity of the slopes, a Student t is used [25]. The Statistic for slopes calculated was 0.597 while the Statistic for slopes tabulated was 2.878 at 18 degrees of freedom and $\alpha = 0.01$ (the probability was 55.8%). Therefore, the slopes are essentially the same.

4. Conclusions

This paper presents an innovative way of obtaining RTP from nafronyl in solution, without any kind of organised media and proposes a new method for direct phosphorimetric determination of nafronyl. This can be a elegant alternative to more sophisticated methodologies such as anti-doping control analysis in sport competition for the analysis of biological samples, previous a detail study of the interferent effects of the sample matrices and to control the concentrations of pharmaceutical preparations, as it has been demonstrated without any matrix effect observed.

The statistical calculation of the assay results showed satisfactory precision of the phosphorimetric method proposed and it has been validated, demonstrating that the Heavy Atoms Induced Room Temperature Phosphorescence can enhance sensitivity and selectivity for the analysis of small amounts of chemicals in real samples.

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