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Selective determination of naproxen in the presence of nonsteroidal anti-inflammatory drugs in serum and urine samples using room temperature liquid phosphorimetry

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Abstract

A very simple, rapid and highly sensitive method is described for determining naproxen in serum and urine. This method is based on room temperature phosphorescence of naproxen in sodium dodecylsulphate micelles, with thallium(I) providing the external heavy atom and sodium sulphite acting as the oxygen scavenger. Under the optimum experimental conditions, the range of application is 0.09–4.5 μg ml^{-1} and the limit of detection is 0.03 μg ml^{-1}. The most relevant characteristic of this method is its great selectivity, e.g. naproxen can be determined in the presence of other nonsteroidal anti-inflammatory drugs (NSAIDs). The clinical applicability of this procedure has been tested, analysing naproxen in serum and urine samples. The analytical recoveries and inter- and intra assay precision data obtained demonstrate the usefulness of this procedure when used with very complex samples.

Keywords: Naproxen; Room temperature phosphorimetry; Serum; Urine

1. Introduction

High sensitivity and selectivity are generally expected in the fluorometric analysis of drugs. However, problems of selectivity and ultimately of sensitivity can occur whilst determining drugs in biological fluids, such as human urine and serum. In such cases, a high and unknown fluorescent background is always present, which can severely reduce the signal-to-noise ratio.

Synchronous fluorescence spectrometric techniques [1–3] have been proposed for the determination of analytes in fluorescent matrices although the results obtained were not good. The combination of synchronous and derivative fluorometry was first suggested by John and Soutar [4] to enhance minor spectral features and to allow more reliable identification of spectra. This technique has made it possible to determine several drugs in serum and urine but its sensitivity is not very high because the samples generally must be diluted in order to lower the interference level from the matrix [5,6]. Better results have

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been achieved by the technique known as matrix isopotential synchronous fluorescence (MISF). This consists of performing synchronous scans through a trajectory, joining points of equal intensity on a fluorescent matrix tridimensional spectrum [7,8].

The selectivity attained in the analysis of drugs using phosphorimetry is much greater than that attained by fluorometry, because not all drugs that fluoresce will phosphoresce and the emission is red-shifted to a less-crowded spectral region. Conventional phosphorimetry at 77 K has rarely been used because of the problems associated with cryogenic conditions. However, these problems have been circumvented by the use of liquid solutions of ordered media, e.g. micelles or cyclodextrins, which are able to provide effective triplet-state protection from quenchers in solution at room temperature.

Several classes of polycyclic aromatic hydrocarbons and drug-related compounds [9] have been found to give micelle stabilized room temperature phosphorescence (MS-RTP) in deoxygenated solutions, using thallium(I) or silver(I) ions as external heavy atoms. Chemical deoxygenation with sodium sulphite is very convenient compared with the use of nitrogen because there is no foaming [10].

Naproxen[NAP:(+)-2-(6-methoxy-2-naphtyl) propionic acid] is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties, which has been employed for the treatment of rheumatoid arthritis and osteoarthritis. NAP is fully absorbed when administered orally and is excreted in the urine as glucuronide or other conjugates. Only a few of the techniques used for determining NAP in biological fluids are useful for both urine and serum samples. These methods include liquid chromatography and gas–liquid chromatography; although such procedures are tedious, time-consuming and require sample pretreatment prior to measurement.

The phosphorescence of NAP at room temperature stabilized by micelles (MSRTP) was reported by Cline Love et al. [11], and a MSRTP method has recently been described for the determination of this drug in pharmaceutical preparations [12]. The combination of MSRTP with the stopped-flow mixing technique has also been used for analysing NAP in serum [13].

In this paper, a nonchromatographic method for the determination of NAP in serum and urine samples is described. This method is based on the room temperature phosphorescence of NAP in aqueous 0.1 mol l^{-1} sodium dodecyl sulphate using thallium(I) as a external heavy atom. The procedure is very sensitive and simple and its clinical applicability is based on the selective determination of NAP in the presence of other nonsteroidal anti-inflammatory drugs (NSAIDs).

2. Experimental

2.1. Reagents

The chemicals used for the preparation of the solutions were of the highest grade available. Demineralized water from a Milli-Q system (Millipore-Ibérica, Madrid, Spain) was used for the preparation of the solutions.

Aqueous stock solutions of 100 μg ml^{-1} NAP (Sigma, Madrid, Spain) were prepared at pH 12. Working standard solutions were prepared by suitable dilution with demineralized water. Stock solutions of 100 μg ml^{-1} NAP were also prepared in 0.1 mol l^{-1} sodium dodecylsulphate (NaDS) (Merck, Darmstadt, Germany). Working standard solutions were prepared by appropriate dilution with 0.1 mol l^{-1} NaDS. Stock solution of 0.5 mol l^{-1} NaDS was prepared by dissolving the required amount in demineralized water. Stock solution of 0.2 mol l^{-1} thallium(I) was prepared by dissolving thallium nitrate (Merck) in demineralized water. Thallium dodecylsulphate was prepared from NaDS and $TlNO_3$ following the procedure described by Cline Love et al. [14].

2.2. Apparatus

All recordings of uncorrected luminescence spectra and measurements of MS-RTP intensities were carried out with a SLM Aminco Bowman (Urbana, IL) series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp and interfaced to a PC 486 microcomputer. Data ac-

quisition and analysis were performed with AB2 software version 2.0 running under OS/2. All measurements were performed in a 10 mm quartz cell and the sample compartment was thermostated at 20 ± 0.5°C.

2.3. General procedure for phosphorescence measurements

The following reagent was added to a 5-ml calibrated flask containing between 0.9 and 45 μg of NAP: 1 ml of 0.5 mol l^{-1} NaDS, 0.5 ml of 0.2 mol l^{-1} sodium sulphite and 1 ml 0.1 mol l^{-1} phosphate buffer pH 7.1 (in that order). The mixture was made up to the mark with demineralized water. The cell was filled with this solution and left for 5 min at room temperature inside the luminescence spectrometer. The cell was then thermostated for 5 min at 20 ± 0.5°C before measuring the phosphorescence signal at 551 nm with excitation at 330 nm.

2.4. Sample preparation

Serum and urine samples were spiked with appropriate amounts of NAP to obtain a final concentration within the range 5–100 μg ml^{-1}. A volume of 100 μl of each serum sample was placed in a test-tube and 1 ml of 0.1 mol l^{-1} trichloroacetic acid was added. The mixture was vortexed for 2 min and centrifuged for 3 min at $4000 \times g$. An appropriate volume of the supernatant was analysed following the general procedure described above. Urine samples were centrifuged for 3 min at $4000 \times g$. An appropriate aliquot of clear supernatant urine was analysed following the general procedure.

3. Results and discussion

Room temperature phosphorescence (RTP) was observed for NAP in a deoxygenated aqueous 0.10 mol l^{-1} TlDS–NaDS (30/70%) micellar solution. Phosphorescence was not measurable when the drug was dissolved in 0.1 mol l^{-1} NaDS and deoxygenated, although intense fluorescence occurred. However, when at least 30% of the sodium counterion was replaced by thallium, there was a dramatic emergence of RTP, with a concomitant decrease in fluorescence intensity. This can be attributed to the greatly increased rate of intersystem crossing from the singlet S_1 to the triplet T_1 and probably to the increased rate of triplet-state radiative deactivation. These two rates are influenced via an effective spin–orbit coupling between the solubilized NAP and the heavy thallium atoms.

Of the NSAIDs listed in Table 1, only NAP phosphoresced at room temperature, resulting in the use of MS-RTP for analysing NAP in the presence of other NSAIDs. In order to establish the best conditions for determining NAP, the effect of variables affecting RTP were studied.

3.1. Effect of pH and sodium sulphite concentration on RTP intensity and deoxygenation time

The phosphorescence intensity of NAP obtained after the system had reached equilibrium was practically pH independent over the range 6.5–8.4. However, the time necessary to reach the equilibrium value increased as the pH was increased from 7.1 to 9.0, because at these values, the deoxygenation rate fell rapidly. At pH 9.0, RTP could only be induced after the solution had been placed in a thermostat at 20 ± 0.5°C for 45 min. Below pH 7.2, the RTP signals appeared immediately on excitation.

The deoxygenation time increased with decreasing concentrations of sodium sulphite. The most intense RTP was obtained with a sodium sulphite concentration over the range 0.02–0.04 mol l^{-1}. At higher concentrations, there was a decrease in phosphorescence intensity due to excess Na^+ displacement of Tl^+ ions from the micellar surface.

Under acidic conditions at $pH < 7$, the reducing ability of the sulphite ion is decreased because of the formation of HSO_3^-. Under basic conditions at $pH > 7.5$, although SO_3^{2-} concentration is higher, the potential of the O_2–H_2O pair is decreased. Therefore, a $pH \leq 7.5$ should be maintained in order to accelerate the deoxygenated step.

Table 1
Phosphorescent characteristics for a NSAID series

Compound[a]	Wavelength of excitation[b] (nm)	Relative fluorescence intensity[c]	Fluorescence quenched[d] (%)	Relative phosphorescence intensity[e]
Naproxen	236, 279, *330*	5.6	98.9	4.3
Diflunisal	314	3.0	5.6	—
Salicylic acid	318	2.5	2.2	—
Acetylsalicylic acid	320	2.3	5.0	—
Acetaminophen	*295*, 336	3.4	3.5	—
Indomethacin	*322*, 330	0.2	8	—
Diclofenac	306	0.9	4	—
Mefenamic acid	357	0.8	0.8	—
Meclofenamic acid	337	2.0	5.4	—
Fluflenamic acid	354	0.9	4.1	—
Piroxicam	320	0.1	5	—

[a] Drug concentration 2 μg ml^{-1}.
[b] Values in italics are maximum excitation wavelengths.
[c] Values obtained in 0.1 mol l^{-1} NaDS micellar solution.
[d] Reflects the decrease in intensity going from NaDS micellar solution to a mixed Tl(I)–NaDS micellar solution.
[e] Values obtained in 0.1 mol l^{-1} NaDS, 0.02 mol l^{-1} Tl(I) and 0.02 mol l^{-1} Na_2SO_3.

A sodium sulphite concentration of 0.02 mol l^{-1} and a pH of 7.1 was selected for the deoxygenation step as a compromise between high luminescence signals and a relatively short time for the appearance of reproducible and convenient phosphorescence intensities.

3.2. Heavy atom and micelle concentration effects

During a systematic survey on the effect different micellar media and heavy atom ions have on the RTP emission of NAP, it was found that Tl(I) and NaDS micelles yielded the greatest RTP intensity and were therefore used for all subsequent experiments.

In a series of experiments with NaDS fixed at 0.1 mol l^{-1} (and that of Na_2SO_3 at 0.02 mol l^{-1} and at pH = 7.1), the concentration of Tl(I) nitrate was varied from 5×10^{-3} to 4×10^{-2} mol l^{-1}. RTP intensity increased with increasing Tl(I) concentrations up to 1.6×10^{-2} mol l^{-1}, above which it remained constant.

Due to the solubility limitations of Tl(I) dodecylsulphate, the influence of micelle concentration was studied for NaDS concentrations in the range 0.01–0.2 mol l^{-1} in the presence of 0.02 mol l^{-1} sodium sulphite and 0.01 mol l^{-1} phosphate buffer (pH = 7.1), at a fixed $[NADS]:[Tl^+] = 2$ ratio for all experiments. The maximum phosphorescence intensities occurred in the range 0.08–0.12 mol l^{-1} NaDS. The concentration selected was 0.1 mol l^{-1}.

3.3. Effect of temperature

Increasing temperature led to lower RTP and accelerated the rate of sulphite oxidation by oxygen. Consequently, the best results were obtained by eliminating the oxygen by reaction with sulphite at room temperature and measuring the phosphorescence at 20 ± 0.5°C.

3.4. Phosphorescence spectral characteristics

Luminescence spectra of NAP are shown in Fig. 1. The spectra were obtained using the instrumental parameters summarized in Table 2 and the experimental conditions described above (0.1 mol l^{-1} NaDS, 0.02 mol l^{-1} $TlNO_3$, 0.02 mol l^{-1} Na_2SO_3 and 0.01 mol l^{-1} phosphate buffer pH 7.1). The residual fluorescence was below 410 nm. The phosphorescence excitation peak was at 330

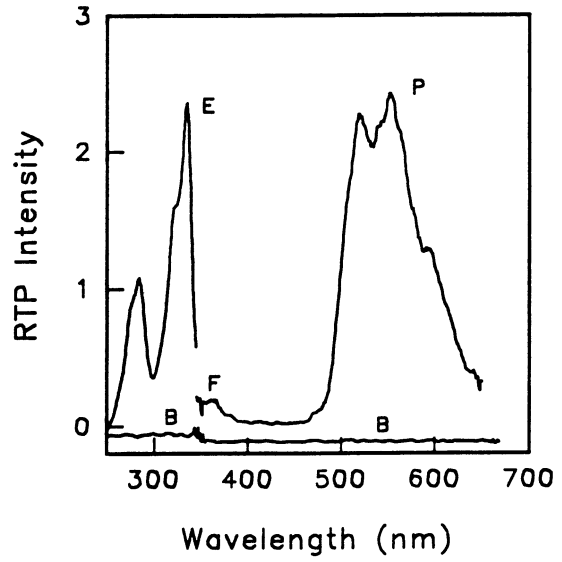

Fig. 1. Luminescence spectra of naproxen. Analyte concentration 1.0 μg ml^{-1}. Other conditions are described in the text. P, RTP; F, residual fluorescence; E, excitation spectrum; B, background.

nm and the emission peaks were at 518 and 550 nm. The phosphorescence lifetime of NAP was approximately 1427 μs.

3.5. Calibration range, sensitivity and precision

Under the conditions recommended in the procedure, there is a linear relationship between phosphorescence intensity and NAP concentration in the range 0.09–4.5 μg ml^{-1}. The correlation coefficient was 0.999 ($n = 11$), indicating excellent linearity. The limit of detection (LOD) was estimated from the equation $C_L = kS_B/m$, where the SD (S_B) from 16 blank determinations, the slope of the calibration curve (m) and $k = 3$ were used [15]. The calculated LOD was 0.03 μg ml^{-1}. The precision of the method was established by repeated assays ($n = 10$) using two different concentrations. The RSD was 2.1 and 1.2% for concentrations of 0.20 and 1.0 μg ml^{-1}, respectively.

Table 2
Optimum instrumental parameters

Excitation/emission slits (nm)	4/8
Minimum period pulse (ms)	10
Scanning speed (nm s^{-1})	2
Decay time (μs)	500
Gate time (μs)	1000
Detector voltage (V)	900

Table 3
Recovery data of NAP added to serum samples

Sample	Concentration (μg ml^{-1})		Recovery (%)
	Added	Found[a]	
1	20.0	19.9	96.5
	30.0	29.2	97.3
	50.0	49.3	98.6
2	20.0	19.4	97.0
	40.0	39.5	98.7
	80.0	80.6	100.7
3	20.0	19.6	98.0
	50.0	50.2	100.4
	100.0	99.1	99.1

[a] Means for four determinations.

3.6. Interferences

The influence of NSAIDs and other drugs was studied by preparing solutions containing 1.0 μg ml^{-1} of NAP and different amounts of the foreign compound. No interference was found for diflunisal, salicylic acid, acetylsalicylic acid, acetaminophen, indometacin, diclofenac, mefenamic acid, meclofenamic acid, saccharin, lactose, lysine, polyethylene glycol, polyvinilpirrolidone at [interferent]:[NAP] ratios up to 300:1. Higher ratios were not assayed. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error less than $\pm 4\%$ in the analytical signal of NAP.

3.7. Determination of NAP added to serum and urine samples

To measure NAP in serum and urine, the standard addition method was used. Analytical recovery experiments with serum and urine samples from healthy volunteers spiked with NAP are shown in Tables 3 and 4, respectively. Most of the concentrations selected are typical of NAP concentrations levels found in human serum and urine during the first 12 h following an oral dose

Table 4
Recovery data of NAP added to urine samples

Sample	Concentration (μg ml^{-1})		Recovery (%)
	Added	Found[a]	
1	5.0	4.8	96.0
	10.0	9.8	98.0
	20.0	19.7	96.5
2	10.0	9.7	97.0
	30.0	29.6	98.6
	50.0	50.2	100.4
3	20.0	20.2	101.0
	40.0	40.3	100.7
	80.0	79.6	99.5

[a] Means for four determinations.

of 500 mg of NAP [16]. The absence of detectable blanks in NAP-free serum and urine samples indicated that the constituents of these biological fluids did not interfere with the phosphorescence measurements.

The within-run precision was assessed by measuring two samples (serum and urine) with different NAP concentrations, four times each. The RSDs for 20 and 50 μg ml^{-1} NAP concentration levels were 2.8 and 2.2% for serum and 2.6 and 2.1% for urine, respectively. The day-to-day reproducibility was studied by analysing, on five different days, ten samples of serum and urine containing 50 μg ml^{-1} of NAP. The RSDs were 3.6% for serum and 3.2% for urine.

4. Conclusions

The study of the RTP of NAP in different micellar media and external heavy atom ions reveals that NaDS and Tl(I) produce the greatest phosphorescence. The chemical deoxygenation of micellar media by using sodium sulphite can be enhanced when the pH is kept under control; hence the time of analysis can be greatly shortened (10 min are required for one determination).

The simplicity of this method, together with the low detection limit, satisfactory recoveries and good selectivity, shows the potential of MS-RTP for routine analysis of NAP in real samples. It is worth noting that the proposed method permits the determination of NAP in urine and serum with excellent results without prior separation steps. In addition, the method is useful for determining NAP in the presence of other NSAIDs.

When this method is compared to the two other phosphorimetric methods [12,13], its sensitivity is generally as good. However, only the proposed procedure has been tested in the presence of NSAIDs and in determining NAP in human urine. The results obtained in the analysis of NAP in biological fluids clearly demonstrates the high selectivity of MS-RTP compared with the other techniques reported for detecting this drug.

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