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Determination of allopurinol by micelle-stabilised room-temperature phosphorescence in real samples

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Abstract

A very simple, rapid and highly sensitive method has been developed for the determination of allopurinol. The method is based on the room temperature phosphorescence of allopurinol in sodium dodecylsulphate (SDS) 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 0.25–7.0 μg ml^{-1} and the limit of detection is 0.014 μg ml^{-1}. The most relevant characteristic of this method is its great selectivity, e.g. allopurinol can be determined in the presence of its metabolite, oxypurinol. The results of the analysis of several pharmaceutical preparations were satisfactory. The clinical applicability of this procedure has been tested by analysing allopurinol in urine samples.

Keywords: Allopurinol; Room temperature phosphorescence; Pharmaceuticals; Urine

1. Introduction

Allopurinol (1H-pyrazolo[3,4-d] pyrimidin-4-ol) is a commonly used drug in the treatment of chronic gout or of hyperuricaemia associated with leukaemia, radiotherapy, antineoplastic agents and treatment with diuretics [1]. Allopurinol and its major metabolite, oxypurinol, are potent inhibitors of xanthine oxidase, the enzyme that converts hypoxanthine to xanthine and xanthine to uric acid.

Various analytical techniques have been employed for the quantitative analysis of allopurinol: UV spectrophotometry [2], photometry using Folin–Ciocalteu reagent [3], fluorimetry based on the quenching effect on the fluorescence of mercurochrome [4], reductive polarographic analysis [5] and flow injection analysis with anodic polarographic detection [6]. Static and flow-through sensors based on xanthine oxidase have been proposed [7,8]. Several chromatographic techniques, such as HPLC using UV detection [9] or voltametric detection [10], gas chromatography [11] and capillary electrophoresis [12,13], have also been proposed. However, these published methods are not always ideal for practical purposes, because they are either unsuitable in the

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Table 1
Characteristics of the methods for allopurinol

Method	Detection	Linearity (μM)	LOD (μM)	Interference of oxypurinol	Reference
Fluorimetry		29–116		Yes	[4]
Polarography		200–1000		Yes	[5]
FIA	Amp	up to 300	1.8	Yes	[6]
Sensor	Amp	0.20–50			[7]
Enzyme	Radiometric	0.1–100		Yes	[8]
HPLC	UV	1.1–150		No	[9]
HPLC	Amp	0.7–700		No	[10]
CE	UV	24–950	5	No	[12]
CE	Amp	0.2–100	0.01	No	[13]
Phosphorimetry		1.8–51.4	0.1	No	This work

presence of oxypurinol or time-consuming, i.e. they contain a derivatisation step, involve arduous sample preparation and need long chromatographic run times. Table 1 summarises some characteristics of these methods.

Phosphorimetry is a selective technique for different organic compounds, such as polycyclic aromatic hydrocarbons, drugs and pesticides. Although fluorimetry is usually more sensitive than phosphorimetry, the broad band of spectra for different compounds limits the selectivity. Therefore, phosphorimetry is often preferred because of its better selectivity than fluorimetry and absorption spectrometry, because not all compounds that fluoresce will phosphoresce and the emission is red-shifted to a less-crowded spectral region. Unfortunately, intense phosphorescence signals seem only to be obtainable if the quenching of the emitting triplet state by oxygen and impurities is prevented, which is not possible for ordinary liquid solutions. For this reason, the application of this technique has been limited, for many years, to rigid glassy solutions at 77 K [14,15].

Room-temperature phosphorimetry (RTP) is very different from the classic low temperature phosphorescence. Since the discovery of RTP, numerous approaches have been developed to induce RTP from various compounds [15–19]. Among them, micelle-stabilised room temperature phosphorescence (MS-RTP) plays a predominant role because of the great enhancement of RTP in solution which can be achieved by incorporating the phosphors into a micellar assembly, using thallium (I) or silver (I) as external heavy atoms in order to increase intersystem crossing [20,21]. Deoxygenation is a key step for observing phosphorescence, while chemical deoxygenation employing sodium sulphite is very convenient compared with bubbling nitrogen because no foaming occurs [22]. It is worth noting that, to our knowledge, there are no RTP methods for determining allopurinol.

RTP is a useful measurement for allopurinol since it is a strong phosphor and oxypurinol does not phosphoresce at all. This latter peculiarity is of interest from an analytical point of view since it permits a sensitive assay to be used in pharmacokinetic and/or clinical studies.

In this paper, a MS-RTP method for the determination of allopurinol is described. The method is based on the RTP of allopurinol in aqueous 0.1 mol l^{-1} sodium dodecylsulphate (SDS) using thallium (I) as an external heavy atom. The procedure is very sensitive and simple and its applicability has been demonstrated in pharmaceutical formulations and urine samples.

2. Experimental

2.1. Reagents

All reagents were of analytical reagent grade and ultrapure water from a Milli-Q plus system (Millipore Ibérica, Madrid, Spain) was used.

Stock standard solution of allopurinol (100 mg l^{-1}) was prepared by dissolving 50 mg of the product (Sigma, Madrid, Spain) in 5 ml of 0.1 mol l^{-1} sodium hydroxide and diluting to 500 ml with 0.1 mol l^{-1} SDS in a calibrated flask. This solution was stable for at least 4 weeks if stored in the dark at 4 °C. More dilute solutions were prepared, when necessary, by diluting with 0.1 mol l^{-1} SDS. Stock solution of 0.5 mol l^{-1} SDS was prepared by dissolving the product from Sigma in ultrapure water. Stock solution of 0.2 mol l^{-1} thallium (I) was prepared by dissolving thallium nitrate (Fluka, Buchs, Switzerland) in ultrapure water. A 0.1 mol l^{-1} sodium sulphite was prepared daily.

2.2. Apparatus

All phosphorimetric measurements were performed on an Aminco Bowman Series 2 luminescence spectrometer, connected to software, which runs on the OS2 operating system. The instrument utilises a 7 W integral pulsed xenon lamp. All measurements were performed in a 10 mm quartz cell and the sample compartment was thermostated at 20 ± 0.5 °C.

2.3. Procedure

For preparation of the calibration graph, an aliquot of allopurinol standard solution containing 2.5–70 μg of the drug was transferred into a 10 ml calibrated flask. Then 2 ml of 0.5 mol l^{-1} SDS, 1 ml of 0.2 mol l^{-1} thallium nitrate, 2 ml of 0.1 mol l^{-1} phosphate buffer (pH 7.0) and 2 ml of 0.1 mol l^{-1} sodium sulphite were successively added and made up to volume with ultrapure water. After thorough mixing, the flask was placed in a water bath at 20 ± 0.5 °C for 5 min. A portion of this solution was transferred into the phosphorescence cuvette and the RTP was measured at 420 nm with excitation at 286 nm.

2.4. Sample preparation

The mixed contents of five pulverised tablets equivalent to 100 mg of allopurinol was accurately weighed and transferred into a 250 ml volumetric flask, to which 1 ml of 0.2 mol l^{-1} sodium hydroxide was added and made to the mark with 0.1 mol l^{-1} SDS. After 5 min in an ultrasonic bath, the suspension was filtered. An aliquot of this solution was diluted ten times with 0.1 mol l^{-1} SDS and analysed following the general procedure.

Urine samples spiked with different amounts of allopurinol were centrifuged at about 1200 × *g* for 5 min. The supernatant was diluted ten times with ultrapure water for analysis.

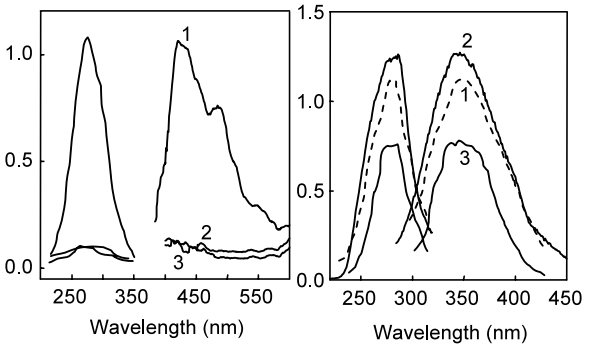

Fig. 1. Luminescence spectra of allopurinol. Left (phosphorescence spectra), (1): Alp + SDS + $TlNO_3$ + Na_2SO_3 + phosphate buffer of pH 7; (2): Alp + SDS + $TlNO_3$ + phosphate buffer; (3): Alp + SDS + Na_2SO_3 + phosphate buffer. Right (fluorescence spectra), (1): Alp + SDS + phosphate buffer; (2): Alp + SDS + Na_2SO_3 + phosphate buffer; (3): Alp + SDS + Na_2SO_3 + Tl (I) + phosphate buffer.

3. Results and discussion

3.1. Spectral characteristics

The fluorescence and RTP spectra of allopurinol are shown in Fig. 1, where it can be seen that RTP signals of allopurinol in SDS micellar solutions can be induced only by using thallium (I) as heavy atom perturber and sodium sulphite as chemical deoxygenator.

The wavelength of maximum phosphorescence emission was red-shifted by 134 nm with respect to the excitation wavelength and by 77 nm with respect to the wavelength of maximum fluorescence emission. In addition, it can be observed that a heavy atom perturber enhanced RTP emission of

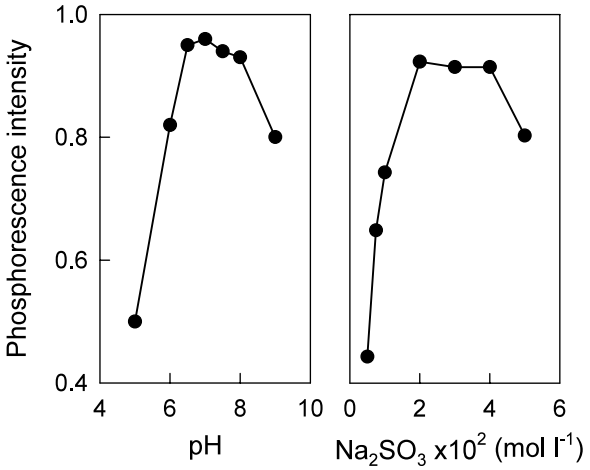

Fig. 2. Influence of (A) pH and (B) sodium sulphite concentration upon phosphorescence intensity.

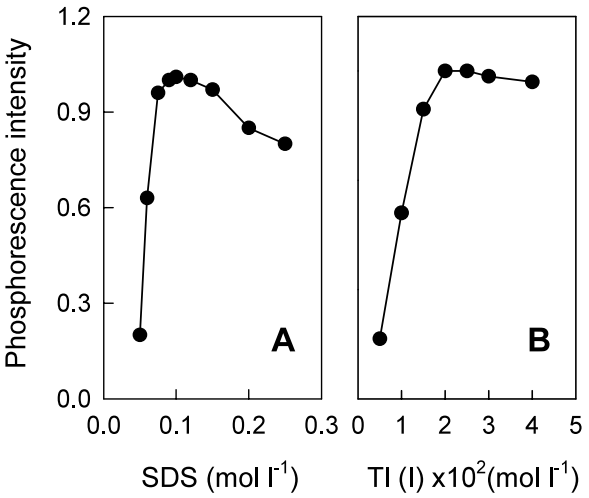

Fig. 3. Effect of (A) SDS concentration and (B) thallium nitrate upon phosphorescence intensity.

allopurinol but quenched its fluorescence, and that the chemical deoxygenator sodium sulphite enhanced the fluorescence intensity of allopurinol.

The phosphorescence lifetime of allopurinol is approximately 400 μs. This is the time required for the population of the excited triplet state to decrease to 1/e of its original value after the excitation source has been turned off.

A solution (blank solution) prepared in the absence of allopurinol and containing SDS, thallium (I) and sulphite did not show any RTP, only background noise, similar to that given by ultrapure water.

The optimum instrumental parameters used in the RTP studies are listed in Table 1.

3.2. Effect of pH and sodium sulphite

The phosphorescence intensity of allopurinol was practically independent over the range 6.5–8.0 (Fig. 2A). However, the time necessary to reach the equilibrium value increased as the pH was increased from 7.5 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 20 min. Below pH 7.5, the RTP signals appeared immediately upon excitation. Therefore, a pH of 7.0 was selected as suitable for the determination. This value was adjusted by adding phosphate buffer solution. The influence of buffer concentration on RTP intensity was also studied. The analytical signal remained constant as buffer concentration increased from 0.01 to 0.1 mol l^{-1} and diminished continuously at higher concentrations. The pH selected was adjusted by the addition of 0.1 mol l^{-1} phosphate buffer of pH 7.0, to obtain suitable sensitivity and buffering capacity. The decrease of RTP with higher concentrations of the buffer can be interpreted as the displacement of Tl^+ from the micelle surface because of the high concentration of Na^+ in the solution.

Sodium sulphite was used to eliminate the oxygen from the micellar solution. The deoxygenation time increased with decreasing concentrations of sodium sulphite. The highest RTP intensity was obtained with a Na_2SO_3 concentration within the range 0.02–0.04 mol l^{-1} (Fig. 2B). At higher concentrations, there was a decrease in RTP intensity, which can also be interpreted as the displacement of Tl^+ by Na^+ from the micelle surface.

3.3. Influence of Tl^+ and SDS concentration

During a systematic survey on the effect of different micellar media and heavy atom ions on the RTP emission of allopurinol, it was found that

Tl^+ and SDS micelles yielded the greatest RTP intensity. They were, therefore, used for all subsequent experiments.

Appreciable stabilisation of the triplet state does not occur at concentrations below the critical micellisation concentration (c.m.c.) and RTP was not observable. Thus, it is imperative to adjust the SDS concentration to well above the c.m.c. Fig. 3A illustrates the influence of SDS concentration upon RTP intensity. For concentrations of the surfactant up to 7.5×10^{-2} mol l^{-1}, a drastic increase in RTP was observed. This is because, at that concentration the c.m.c. of the mixed system, SDS–thallium dodecylsulphate (TlDS) is reached.

Thallium (I) produces an effective spinorbital coupling, facilitating the intersystem crossing between singlet and triplet states that permits the phosphorescence emission. Fig. 3B shows that RTP intensity increased with increasing Tl^+ concentration up to 2×10^{-2} mol l^{-1}, above which it remained constant.

Insolubility phenomena, due to TlDS precipitation, were observed at concentrations of SDS higher than 0.20 mol l^{-1} or Tl^+ higher than 4×10^{-2} mol l^{-1}. According to the above measurements, the concentrations selected were 0.10 mol l^{-1} for SDS and 2×10^{-2} mol l^{-1} for thallium nitrate.

3.4. Effect of temperature

The RTP intensities decreased with increases in temperature, while the residual fluorescence intensities decreased slightly with increases in temperature. These effects are mainly related to molecular thermal motion and intermolecular energy conversion, the former causing collisional deactivation of the phosphor. A temperature of 20 ± 0.5 °C was selected.

3.5. Features of the proposed method

Under the instrumental and chemical operating conditions used, there was a linear relationship between RTP intensity and allopurinol concentration in the range 0.25–7.0 μg ml^{-1} (2×10^{-6}–5×10^{-5} mol l^{-1}). The regression equation was:

Table 2
Optimum instrumental parameters

Fluorescence wavelength (ex/em)	286/343 nm
Phosphorescence wavelength (ex/em)	286/420 nm
Scanning speed	2 nm s^{-1}
Minimum period pulse	10 ms
Delay time	300 μs
Gate width	1500 μs
Detector voltage	1000 V

$$I_P = (0.212 \pm 0.013) + (0.148 \pm 0.004) \cdot C$$

where I_P is the RTP intensity and C the concentration of allopurinol in μg ml^{-1}. The correlation coefficient was 0.999 ($n = 11$), indicating excellent linearity. The detection limit, estimated according to IUPAC recommendations [23], was 0.014 μg ml^{-1}. The repeatability was evaluated at two analyte concentrations, 0.95 and 5.4 μg ml^{-1}, for which the relative standard deviations (R.S.D.) ($n = 10$ for each level) were 0.41 and 0.36%, respectively. The reproducibility of the method was studied by analysing, on 5 different days, six identical solutions of allopurinol (3 μg ml^{-1}). Every day three determinations of each solution were made; the R.S.D. was 1.6%.

3.6. Interferences

The influence of foreign species was studied by preparing solutions containing 0.7 μg ml^{-1} of allopurinol and increasing concentrations of the potential interferent up to 210 μg ml^{-1}. The tolerance of each foreign species was taken as the largest amount yielding a variation in the phosphorescence intensity of allopurinol of less than ± 4%.

No interference was found for lactose, starch, magnesium stearate, oxalate, diclofenac, ascorbic acid, benzbromarone, mefenamic acid or acetaminophen (300-fold excess was the maximum tested), nor for a 200-fold excess of sorbic acid, sodium citrate, polyvinylpyrrolidone, polyethylene glycol and saccharin, a 100-fold excess of salicylic acid, acetylsalicylic acid, indomethacin, diflunisal and oxypurinol or a 50-fold excess of caffeine.

Table 3
Determination of allopurinol in pharmaceutical preparations

Sample (supplier)	Nominal content (mg/tablet)	Proposed method[a]	Reference method[b]
Zyloric (Faes)	100	99.8±1.3	99.5
Acifugan (Lácer)	100	99.7±2.0	100.7
Facilit (Fides Ecopharma)	100	100.1±1.5	99.2

[a] Mean of four determinations±S.D.
[b] UV spectrophotometric method. Values are the mean of two determinations.

Table 4
Recovery of allopurinol in pharmaceutical preparations

Sample (supplier)	Amount added (mg)	Amount found[a] (mg)	Recovery (%)
Zyloric (Faes)	20	19.6±1.1	98.0±5.5
	80	79.4±1.0	99.3±1.3
Acifugan (Lácer)	20	18.6±1.5	93.3±7.5
	80	78.9±1.2	98.6±1.5
Facilit (Fides Ecopharma)	20	19.1±1.3	95.5±6.5
	80	76.2±1.3	95.2±1.6

[a] Mean of four determinations±S.D.

Table 5
Recovery data of allopurinol added to urine samples

Sample	Concentration (μg ml^{-1})		Recovery (%)
	Added	Found[a]	
I	7.0	6.9±0.4	98.6±5.7
	10.0	10.0±0.3	100.0±3.0
	15.0	14.8±0.3	98.7±2.0
II	8.0	8.0±0.5	100.1±6.3
	10.0	9.9±0.5	99.5±5.0
	15.0	14.9±0.4	99.4±2.7
III	10.0	9.9±0.4	99.7±4.0
	15.0	14.8±0.3	98.5±2.0
	20.0	19.7±0.3	98.3±1.5

[a] Mean of four determinations±S.D.

3.7. Applications

The present method has great potential for the sensitive and rapid determination of allopurinol in real samples. This was confirmed by the results obtained for the determination of allopurinol in pharmaceutical formulations and urine samples. Three different pharmaceutical formulations were analysed. The data in Table 2 show that the assay results were in good agreement with the pharmacopeia reference procedure and the labelled contents.

The recovery was determined by adding various amounts of allopurinol to each pharmaceutical preparation and subtracting the results obtained for pharmaceuticals to which no allopurinol has been added. Taking into account that recoveries obtained were close to 100%, it may be assumed that no interfering substances were encountered (Table 3).

The determination of allopurinol in urine was investigated using human urine. Tables 4 and 5 summarise the results obtained by adding different amounts (between 7 and 20 μg) of allopurinol per ml of urine and diluting ten times prior to application of the proposed procedure. As can be seen the recoveries were within the range 98–100% of the amounts added in the preparation of synthetic samples. It must be emphasised that the procedure is useful in clinical studies because the metabolite of allopurinol, oxypurinol, does not act as a phosphor at room temperature.

4. Conclusions

An RTP method for the direct determination of allopurinol in pharmaceutical preparations and urine has been developed. The determination can be performed by measuring phosphorescence intensity at 420 nm with excitation at 286 nm with excellent precision and sensitivity.

Owing to the high selectivity of the phosphorimetric methods, the proposed procedure shows no significant interferences and is suitable for the determination of the drug in pharmaceutical dosage forms.

The method is very attractive for the determination of allopurinol in urine because its metabolite, oxypurinol, is a non interferent since it does not phosphoresce. It represents a useful procedure for routine analytical work and pharmacokinetic studies.

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