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Flow-injection fluorimetric determination of penicillamine and tiopronin in pharmaceutical preparations

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Abstract

Two flow-injection methods for the fluorimetric determination of penicillamine and tiopronin are proposed. The procedures are based on the oxidation of these drugs by thallium(III). In hydrochloric acid medium the fluorescence of thallium(I) formed in the oxidation of penicillamine or tiopronin is monitored using excitation and emission wavelengths of $\lambda_{ex} = 227$ nm and $\lambda_{em} = 419$ nm respectively. Linear calibration graphs were obtained between 3×10^{-7} and 8×10^{-6} M for penicillamine and between 8×10^{-7} and 2×10^{-5} M for tiopronin with sampling frequencies of 90 and 45 samples h^{-1} respectively. The relative standard deviations were in the ranges 0.48–0.29% for penicillamine and 1.04–0.31% for tiopronin. The applicability of the method to the determination of both drugs in pharmaceutical preparations was demonstrated by investigating the effect of potential interferences and by analysis of commercial preparations.

Keywords: Flow-injection; Penicillamine; Pharmaceuticals; Thallium(I) fluorescence; Tiopronin

1. Introduction

Thiol-containing drugs are incorporated as therapeutic agents in a variety of pharmaceutical preparations. Among these drugs tiopronin(*N*-2-mercaptopropionylglycine) and penicillamine (3-mercapto-D-valine) have been frequently used, the former in the treatment of liver disorders and eczematous skin diseases and the latter drug to treat Wilson's disease and rheumatoid arthritis [1]. Both drugs are efficient antidotes to heavy metal poisoning.

Ellman's assay is the most popular batch assay for quantitation of thiols [2]. This based on thiol–disulphide interchange: the reaction of thiolate anion with excess Ellman's reagent [5-5′-dithiobis(2-nitrobenzoic acid)] at pH 7–8 favours the stoichiometric formation of a mixed disulphide and 2-thio-2-nitrobenzoate. The large molar absorptivity of this compound makes a useful spectrophotometric assay of thiol at concentration levels higher than 3×10^{-6} M. Penicillamine and tiopronin have been determined by spectrophotometry through the formation of their complexes

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with metal ions, mainly Co(II) and Pd(II) [3,4]. Spectrofluorimetric [5], potentiometric [6] and voltametric [7] procedures have also been proposed.

In recent years, flow-injection (FI) analysis has found application in the determination of both drugs. FI procedures generally involve the formation of complexes between the drugs and Co(II), Pd(II) or Cu(II) which are monitored by spectrophotometry [8–10]. Another approach makes use of the formation of *S*-nitrosothiol in the reaction between penicillamine and nitrous acid; the *S*-nitrosothiol is hydrolysed, with the subsequent formation of an azo dye which is monitored [11]. Finally, the inhibitory effects of these drugs on the oxidation of thiamine to fluorescent thiochrome by mercury(II) [12] or on the chemiluminescent hypochlorite–luminol reaction catalysed by Cu(II) [13,14] have also been adapted to flow configurations. However, none of these FI procedures allows accurate determination of drugs at concentration levels less than 1.0×10^{-5} M.

There is still a need for a sensitive yet simple and rapid method for the analysis of penicillamine or tiopronin which is capable of being used with a large number of samples (possibly adaptable to automation). The above methods do not completely satisfy these criteria especially when the level of the drug is very low (10^{-6} M or less). Therefore, a FI procedure was developed based on the oxidation of both drugs by thallium(III), the parameter monitored being the fluorescence of thallium(I) formed. The procedure is very simple, inexpensive and rapid and has been applied satisfactorily to the determination of penicillamine or tiopronin in pharmaceutical preparations.

2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade and the solutions were prepared with doubly-distilled water. Penicillamine (Fluka, Switzerland) and tiopronin (Sigma, St. Louis, MO), 1×10^{-3} M stock standard solutions, were prepared in water and kept in dark bottles at 4°C. Working solutions of lower concentration were prepared daily by appropriate dilution of the stock standard solutions with water.

Thallium(III) stock solution (1×10^{-3} M) in 0.2 M hydrochloric acid was prepared from thallium(III) nitrate trihydrate (Merck, Darmstadt, Germany). Solutions of lower concentration were prepared by dilution with water.

2.2. Apparatus

A SLM–Aminco Bowman (Urban, IL) Series 2 spectrofluorimeter was used for recording spectra; excitation and emission spectra were corrected. The detector used in the flow systems was a Perkin-Elmer (Norwalk, CT) model 3000 spectrofluorimeter with a Hellma (Jamaica, NY) 176.052 QS flow cell (pathlength 1 cm and inner volume 25 μl) connected to a Linseis (Selb, Germany) 6215 recorder. A Gilson (Villier-le-Bel, France) Miniplus-4 peristaltic pump, an Omnifit (Cambridge, UK) rotary valve and various end fittings and connectors (Omnifit) were also used. With the exception of the pump tube (Tygon), PTFE tubing (0.5 mm i.d.) was used throughout the manifold.

2.3. Manifold

A schematic diagram of the instrumental set-up is shown in Fig. 1. The sample is introduced at point S with the aid of the Omnifit rotary valve. The reagent streams are pumped at the same flow

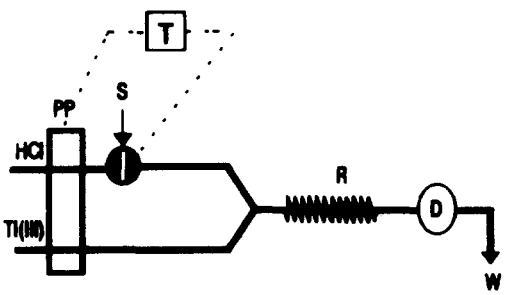

Fig. 1. Schematic diagram of the FI system for fluorimetric determination of penicillamine or tiopronin: (PP) peristaltic pump; (S) point of sample injection; (R) reaction coil; (D) fluorimeter; (W) waste; (T) timer (for tiopronin only).

rate in order to achieve effective mixing of both solutions. The reaction is started by injection of the sample. For the determination of penicillamine, when the sample zone reaches the flow cell the fluorescence of thallium(I) formed is measured ($\lambda_{ex} = 227$ nm; $\lambda_{em} = 419$ nm).

For tiopronin determination, a laboratory-built timer (T) was used to control the pump and the injection valve in order to achieve a longer residence time. The timer was programmed so that when the sample zone was in the reaction coil the flow was stopped.

2.4. Determination of penicillamine and tiopronin in pharmaceutical preparations

The tablets or capsules (five or more) were finely powdered. A portion of this powder was accurately weighed and dissolved in water and diluted to 500 ml in a calibrated flask. This solution was diluted appropriately with water, aspirated into the sample loop by means of the peristaltic pump and injected into the FI system.

3. Results and discussion

Penicillamine and tiopronin are oxidized by thallium(III) in acidic solutions to form fluorescent thallium(I). This violet fluorescence has been attributed to the existence of $TlCl_3^{2-}$ anionic complex [15] and hence the reaction is carried out in the presence of hydrochloric acid. The fluorescence spectra of a solution containing 1×10^{-4} M Tl(III), 5×10^{-6} M penicillamine and 1 M HCl are exactly superimposable on that of Tl(I) in 1 M HCl (Fig. 2). The excitation maximum occurs at 227 nm and the fluorescence emission maximum at 419 nm.

The fluorescence intensity increases with increasing chloride concentrations up to about 0.9 M, above which it remains constant. 1.0 M hydrochloric acid was selected for further investigations.

Both drugs can also be oxidized in a flow system by injection of their solutions into an inert carrier (HCl) stream, which is then mixed with a stream of thallium(III).

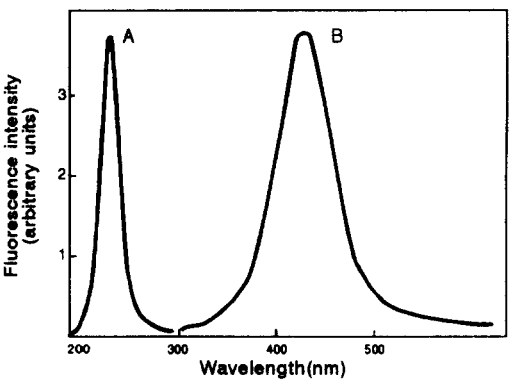

Fig. 2. Excitation (A) and emission (B) spectra. [Tl(III)] = 1×10^{-4} M; [penicillamine] = 5×10^{-6} M; [HCl] = 1 M.

Several experiments were performed to establish the optimum conditions for the FI determination of penicillamine and tiopronin. Hydrodynamic and chemical variables were studies using the manifold depicted in Fig. 1.

3.1. Influence of manifold parameters

The variables studied for optimization of the mainfold parameters were sample volume injected, flow rate and reaction coil.

The reagent concentrations used in these experiments was as follows: thallium(III) line, 1×10^{-4} M; hydrochloric acid line, 1 M. The mixing ratio between both streams was always 1:1 and the sample solution (penicillamine or tiopronin) was 5×10^{-6} M.

The sample volume was varied between 35 and 235 μl. An increase in loop size produced an increase in peak height up to 95 μl for penicillamine and 170 μl for tiopronin, above which the signal decreased slightly. Sample volumes of 95 and 170 μl for penicillamine and tiopronin respectively were chosen for further experiments.

The peak height obviously depends on the residence time of the sample zone in the system, i.e. on the total flow rate and the tube length. The effect on the flow rate was checked over the range 0.17–1.75 ml min^{-1}. The lower flow rates gave higher fluorescence intensities although, up to 0.7 ml min^{-1}, peak height reproducibility was poor and the peaks were so broad that sample through-

put was very low. A flow rate of 1.0 ml min^{-1} (0.5 ml min^{-1} for each channel) was selected as the best compromise between reproducibility, sensitivity and throughput.

For penicillamine there was little increase in the fluorescence intensity with increased reactor lengths up to 100 cm, above which the signal remained virtually constant. The length chosen for the reactor was 100 cm. For tiopronin the peak height increased continuously with increasing reactor length, i.e. residence time, because its oxidation by Tl(III) is not very fast. The best results were obtained if the pump was stopped when the sample plug was located in the reaction coil, R. A coil length of 100 cm and a timer programmed to ensure that 10 s after the sample injection the flow stops for 60 s were chosen as a compromise between analytical signal and sampling rate.

3.2. Influence of reagent concentrations

The effects of varying concentrations of thallium(III) and hydrochloric acid were tested in the optimized flow system.

The influence of the concentration of hydrochloric acid solution (carrier) on the peak height is shown in Fig. 3. The carrier selected was 1.5 M HCl for penicillamine and 1.0 M HCl for tiopronin in order to obtain the highest value of fluorescence intensity.

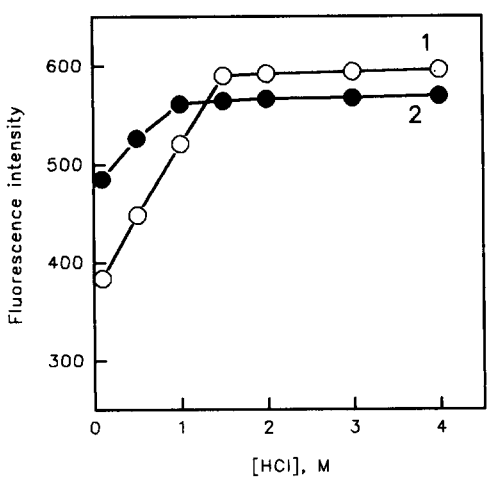

Fig. 3. Effect of hydrochloric acid concentration on the peak height: (1) penicillamine; (2) tiopronin.

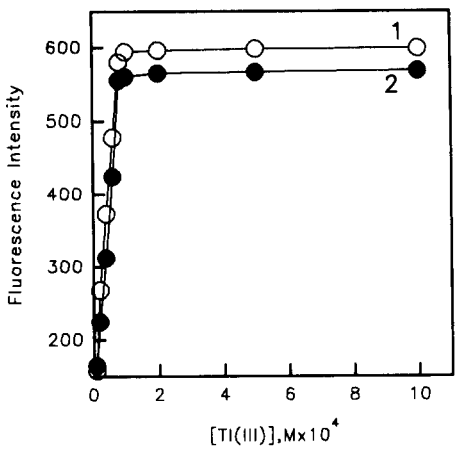

Fig. 4. Effect of thallium(III) concentration on the peak height: (1) penicillamine; (2) tiopronin.

The peak height for both drugs increased steeply with an increase in thallium(III) concentration up to 8×10^{-5} M and was constant at higher concentrations (Fig. 4). The concentration selected in the determination of both drugs was 2×10^{-4} M.

3.3. Calibration graphs and statistical data

A series of standard sample solutions (at least 11 samples covering the whole range of concentrations) was injected into the manifold under selected conditions to test the linearity of the calibration graphs for penicillamine and tiopronin. A linear relationship between drug concentration and fluorescence intensity was obtained in the ranges 3×10^{-7}–8.0×10^{-6} M for penicillamine and 8×10^{-7}–2.0×10^{-5} M for tiopronin. The regression equations of the calibration graphs are:

$$I_F = (115.7 \pm 1.6) \times 10^6[\text{penicillamine (M)}] + (14 \pm 1.1), \quad r = 0.9992$$

$$I_F = (109.7 \pm 2.1) \times 10^6[\text{tiopronin (M)}] + (12 \pm 1.9), \quad r = 0.9989$$

The sampling rate was 90 samples h^{-1} for penicillamine and 45 samples h^{-1} for tiopronin.

The precision was tested by injecting 10 samples of each analyte at two concentration levels. The relative standard deviations were 0.48% and 1.04% at the 1.0×10^{-6} M level and 0.29% and 0.31% at the 6×10^{-6} M level for penicillamine and tiopronin respectively.

The detection limits calculated according to IUPAC recommendations [16] were 6.2×10^{-8} M for penicillamine and 9.8×10^{-8} M for tiopronin.

3.4. Study of possible sources of interference

The effect of foreign species on both compounds was studied. The results for the determination of 5×10^{-6} M penicillamine and tiopronin are listed in Table 1. Since the aim of this work is the determination of these drugs in pharmaceutical preparations, the effect of common excipients and additives was carefully considered. The tolerance limit was taken as the concentration causing an error of not more than $\pm 3\%$ in the determination of each drug. As can be seen, the proposed methods are sufficiently selective.

Table 1
Effect of various foreign species on the determination of 6×10^{-6} M penicillamine or tiopronin

Foreign species	Maximum tolerable molar ratio ([species]/[drug])
Caffeine, glucose, galactose, saccharose, fructose, alanine, maltose, citrate, tartrate, calcium (II)	100[a]
Lactose	50
Benzoic acid, magnesium (II), cystine, hippuric acid	20
Acetylsalicylic acid, uric acid	1
Cysteine	0.2
Ascorbic acid[b]	0.1 (40)[b]

[a] Maximum molar ratio tested.
[b] Ascorbic acid interference was eliminated by heating the solution in alkaline medium at 50°C for 30 min.

Table 2
Determination of penicillamine and tiopronin in pharmaceutical preparations

Sample[a] (laboratory)	Drug	Content (mg per capsule/tablet)	
		FI method[b]	Certified
Cupripen capsules (Rubio)	Penicillamine	248.7 ± 1.1	250
Sufortanon tablet (Sargent)	Penicillamine	248.2 ± 1.2	250
Hepadigest tablet (Uriach)	Tiopronin	99.1 ± 1.9	100
Sutilan tablet (Cusi)	Tiopronin	98.7 ± 1.2	100

[a] Composition of samples: Cupripen: 250 mg penicillamine, excipient; Sufortanon: 250 mg penicillamine, excipient; Hepadigest: 100 mg tiopronin, 10 mg meto-clopamide hydrocloride, 100 mg cyclobutyrol calcium, excipient; Sutilan: 100 mg tiopronin, excipient.
[b] Means of four determinations ± SD.

3.5. Applications

The proposed FI method for penicillamine and tiopronin was applied to the determination of both drugs in various pharmaceutical preparations. The results obtained and the labelled contents are summarized in Table 2. There were no significant differences between labelled contents and those obtained by the proposed method for each drug.

Recovery studies were also performed on each of the analyzed samples by adding a known amount of penicillamine or tiopronin to the sample before the recommended treatment. Recoveries ranged from 98.1–101.8%. Table 3 summarizes some of the results obtained.

4. Conclusions

A comparison of the proposed FI fluorimetric methods with other existing methods shows ad-

Table 3
Recoveries of the drugs added to samples

Sample	Added (mg)	Found (mg)	Recovery (%)
Cupripen	50	49.05	98.1
	100	101.80	101.8
	150	151.80	101.2
Sufortanon	50	50.75	101.5
	100	100.80	100.8
	150	148.95	99.3
Hepadigest	50	49.25	98.5
	100	101.60	101.6
	150	149.10	99.4
Sutilan	50	19.20	98.4
	100	99.50	99.5
	200	201.80	100.9

vantages as regards simplicity and sensitivity. The simplicity of sample preparation and the automatic control of time using the FI system means a less expensive and more versatile system with considerably reduced analysis times compared with those of the reported manual methods. In addition, the sensitivity of the method is higher (about two decades) than that obtained using the reported FI procedures [8–14]. Linearity, precision and recovery are also satisfactory. In summary, the method described here is simple, highly sensitive and useful for the routine quantitative analysis of penicillamine and tiopronin in pharmaceutical samples with minimal sample conditioning and can be used as an assay for dosage forms, since there is no significant interference from potential excipients. However, the possibility of interference by degradation products cannot be excluded and would need further examination.

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