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Determination of penicillamine or tiopronin in pharmaceutical preparations by flow injection analysis

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Abstract: Two flow injection analysis (FIA) methods, using spectrophotometric detection, are proposed for the determination of penicillamine or tiopronin [*N*-(2-mercaptopropionylglycine)]. The procedures are based on the formation of yellow complexes between these thiol-containing drugs and Pd(II), in a 1 M or 0.25 M HCl medium, respectively. With peak height as a quantitative parameter, penicillamine is determined over the range 1.0×10^{-5}–7.0×10^{-4} M; for tiopronin the range is 1.0×10^{-5}–6.0×10^{-4} M. The methods have been applied to the routine determination of the drugs in pharmaceutical preparations.

Keywords: *Penicillamine; tiopronin; palladium (II); FIA; pharmaceutical preparations.*

Introduction

Thiol-containing compounds are frequently used as therapeutic agents. Among these drugs, penicillamine and tiopronin are commonly used. The former has been used to treat Wilson's disease and rheumatoid arthritis [1]; the latter drug has also been found to have applications in eczematous skin diseases and in the treatment of liver disorders [2]. Both drugs are efficient antidotes in heavy metal poisoning [1–3].

These drugs have been determined by chromatographic [4–8], spectrophotometric [9–12], spectrofluorimetric [11], potentiometric [13–15], voltammetric [16], NMR [17–18] and radioimmunoassay [19] procedures. Reviews of methods for penicillamine determination have also been published [20–21]. However, only two studies on the determination of penicillamine have used flow injection techniques [22, 23]. The formation of the penicillamine complex with Co(II) in an ammonium acetate medium is used in one of these methods [22]; the other method is based on the formation of *S*-nitrosothiol in the reaction between penicillamine and nitrous acid. This is followed by the hydrolysis of the *S*-nitrosothiol with Hg(II) and subsequent formation of an azo-dye [23]. For the determination of tiopronin by FIA [24, 25], one of the methods proposed is based on the formation of a complex at pH 10 with Ni(II) [24]. The other method is based on the inhibitory effect of tiopronin on the oxidation of thiamine to thiochrome by Hg(II) [25].

The aim of the present work was to develop two simple and fast methods for the routine determination of penicillamine or tiopronin in pharmaceutical preparations. The procedures proposed are based on the formation of yellow complexes between penicillamine or tiopronin with Pd(II). The FIA technique is used and the peak height is taken as a quantitative parameter.

Experimental

Apparatus

The FIA system comprised a Gilson HP4 peristaltic pump (Worthington, OH, USA), an Omnifit injection valve (NY, USA) a Hellma 18-μl flow cell (Jamaica, NY, USA) and a Pye Unicam spectrophotometer SP8-200 UV–vis (Cambridge, UK) as the detector. Connecting tubing (0.5-mm bore), poly(tetrafluoroethylene) (PTFE) tubing and various end-fittings and connectors (Omnifit) were used.

Reagents

All chemicals were of analytical-reagent

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grade and the solutions were prepared with double-distilled water.

Palladium dichloride standard solution (5×10^{-3} M). The standard solution was prepared by dissolving 0.2216 g of $PdCl_2$ (Merck) in 5 ml of water, to which 0.5 ml of concentrated HCl had been added, and warming the mixture in a water bath. The solution was cooled and diluted with water in a 250-ml calibrated flask.

More dilute solutions were obtained by appropriate dilution with water.

Hydrochloric acid (6 M). This was prepared by dilution of the concentrated acid.

Stock D-penicillamine solution (1×10^{-3} M). The stock solution of D-penicillamine was prepared daily by dissolving 0.0149 g of penicillamine (Fluka, Switzerland) in 100 ml of water; the stock solution was kept at 4°C.

Stock tiopronin solution (1×10^{-3} M). The stock solution of tiopronin was prepared daily by dissolving 0.0163 g of tiopronin [*N*-(2-mercaptopropionylglycine)] (Fluka, Switzerland) in 100 ml of water; the stock solution was kept at 4°C.

Procedure for calibration

Using the FIA manifold shown in Fig. 1, 70 µl of penicillamine or tiopronin solution was injected into an inert carrier stream, which then joined the reagent stream (1×10^{-3} M $PdCl_2$ in 1 M HCl for penicillamine, 5×10^{-4} M $PdCl_2$ in 0.25 M HCl for tiopronin); the peak height was measured at 400 nm. A calibration graph was prepared by plotting the peak height (h) versus penicillamine or tiopronin concentration.

Determination of penicillamine in pharmaceutical preparations

Cupripen capsules. The contents of at least 30 capsules were weighed and mixed to obtain the mean weight per capsule. An accurately weighed portion (10–20 mg) of the powder from the capsules was dissolved in water and diluted to 250 ml in a calibrated flask. A suitable aliquot was analysed by the FIA procedure.

Surfotanon tablets. At least 30 tablets were weighed to obtain the mean weight per tablet. An accurately weighed tablet was dissolved in water and diluted to 250 ml in a calibrated flask. The solution was filtered and diluted appropriately with water; a suitable aliquot was analysed by the FIA procedure.

Determination of tiopronin in pharmaceutical preparations

At least 30 tablets of Sutilan or Hepadigest were weighed to obtain the mean weight per tablet. An accurately weighed tablet was dissolved in water and diluted to 200 ml in a calibrated flask. The solution was filtered and diluted appropriately with water; a suitable aliquot was analysed by the FIA procedure.

Results and Discussion

Penicillamine and tiopronin react with Pd(II) to produce yellow complexes; this reaction has been used by Raggi and others [10] for the spectrophotometric determination of these drugs and by Akerfeld and others [12] for the spectrophotometric determination of penicillamine only. The present authors have demonstrated that both complexes show a drug–Pd(II) stoichiometric ratio of 2:1 with absorption maxima at about 400 nm in the hydrochloric acid medium used in these methods. Penicillamine and tiopronin do not absorb at this wavelength and palladium(II) chloride has a very low absorbance under the same experimental conditions.

The reactions between Pd(II) and penicillamine or tiopronin have been adapted in order to develop two spectrophotometric–FIA methods for determining the drugs.

The design of the manifold shown in Fig. 1 is simple. The sample is injected into a water

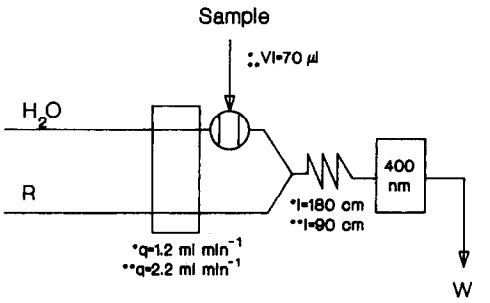

Figure 1
FIA manifold for the determination of penicillamine or tiopronin. R = reagent solutions: 1×10^{-3} M Pd(II) in 1 M HCl for penicillamine and 5×10^{-4} M Pd(II) in 0.25 M HCl for tiopronin. V_i = volume injected (loop size); l = length of reaction coil; and q = flow rate (pump). *Penicillamine, **tiopronin.

stream, which is then mixed with a stream of palladium(II) chloride dissolved in hydrochloric acid. Pd(II) forms the penicillamine–Pd(II) or tiopronin–Pd(II) complex and the absorbance is measured by means of the detector at 400 nm. In the absence of the drug (blank) a very small signal is obtained. The presence of penicillamine or tiopronin causes an increase in the analytical signal, which is proportional to the drug concentration.

The influence of FIA and chemical variables have been studied in order to establish FIA methods for penicillamine and tiopronin determination.

Figures 2 and 3 show the effects of the loop size, reactor length and flow rate on the peak height for penicillamine and tiopronin, respectively. An increase in loop size produces

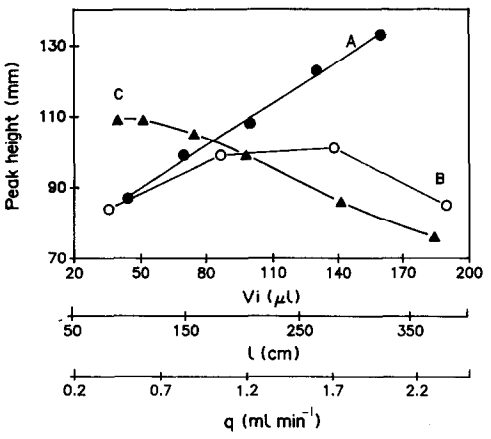

Figure 2
Effect of A, loop size; B, reactor length; and C, pumping rate, on the peak height. Sample injected = 5.0×10^{-4} M penicillamine.

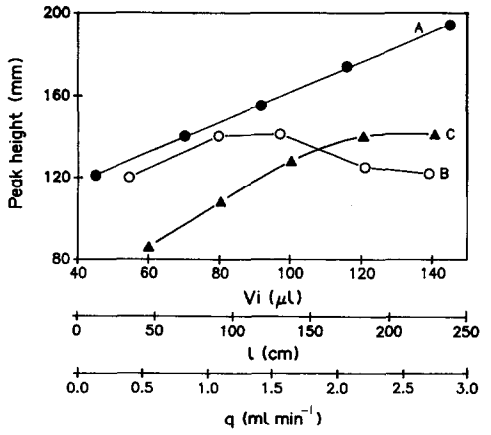

Figure 3
Effect of A, loop size; B, reactor length; and C pumping rate, on the peak height. Sample injected = 5.0×10^{-4} M tiopronin.

an increase in peak height in both cases [Figs 2(A) and 3(A)]. A loop size of 70 μl was chosen, a volume at which an acceptable sensitivity is obtained with no excessive waste of sample.

The influence of reactor length was studied from the minimum distance possible between the injection valve and detector up to 4 m. The results [Figs 2(B) and 3(B)] showed that the peak height increased as the reactor length increased. However, when a certain reactor length was reached, the peak height decreased. At intermediate lengths, peak heights remained constant. Reactor lengths of 180 cm or 90 cm (i.d. 0.5 mm) were selected as these provided the greatest reproducibility for penicillamine and tiopronin, respectively.

The effects of flow rate on peak height was studied over the range 0.5–2.7 ml min^{-1} with the two compounds. The results obtained are shown in Figs 2(C) and 3(C). For penicillamine a flow rate of 1.2 ml min^{-1} was selected; a flow rate of 2.2 ml min^{-1} was used with tiopronin.

It is known that in neutral or slightly acid solutions Pd(II) ions yield coloured complexes with a number of compounds. In acidic solution, however, the reaction is much more specific for compounds containing sulphur [12] and this is the case with penicillamine and tiopronin. For this reason an acidic medium was selected, the best results being obtained with 1 M HCl for penicillamine and 0.25 M HCl for tiopronin, as can be seen in Fig. 4. These experiments were carried out under the FIA experimental conditions selected.

The influence of Pd(II) concentration was studied in the 1.0×10^{-4}–1.5×10^{-3} M range with a fixed 5.0×10^{-4} M concentration of penicillamine or tiopronin in a medium of 1 M or 0.25 M HCl, respectively, under the FIA experimental conditions selected. As can be seen in Fig. 5, constant and maximum values of peak heights are obtained with Pd(II) concentrations higher than 7.5×10^{-4} M and 4×10^{-4} M for penicillamine and tiopronin, respectively. Concentrations of 1.0×10^{-3} M $PdCl_2$ for penicillamine and 5.0×10^{-4} M for tiopronin were selected, which were sufficient for the total formation of the complexes in the ranges of the calibration graphs used in the determination of both compounds.

Determination of penicillamine or tiopronin

With the described manifold and under the selected experimental conditions ($1.0 \times$

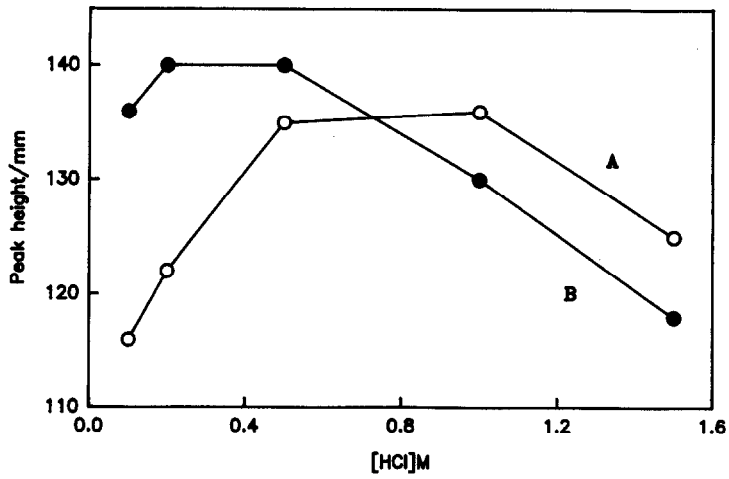

Figure 4
Effect of HCl concentration on peak height. Curve A: 5×10^{-4} M penicillamine; 1×10^{-3} M Pd(II); V_i = 70 μl; l = 180 cm; and q = 1.2 ml min^{-1}. Curve B: 5×10^{-4} M tiopronin; 5×10^{-4} M Pd(II); V_i = 70 μl; l = 90 cm; and q = 2.2 ml min^{-1}.

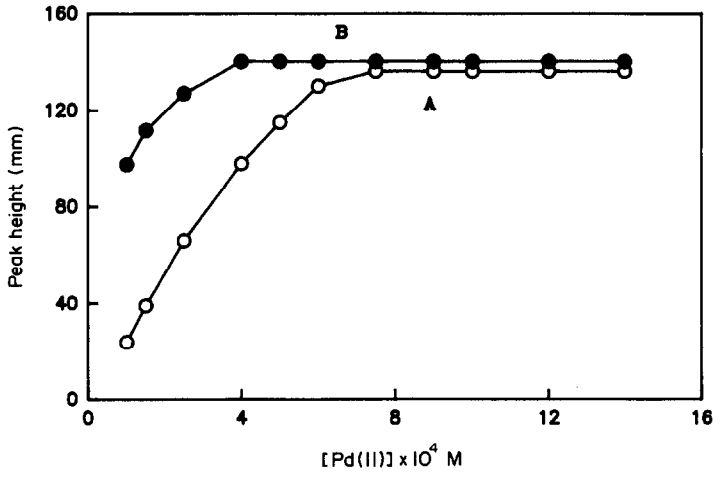

Figure 5
Effect of Pd(II) concentration on peak height. Curve A: 5×10^{-4} M penicillamine; 1 M HCl; V_i = 70 μl; l = 180 cm; and q = 1.2 ml min^{-1}. Curve B: 5×10^{-4} M tiopronin; 0.25 M HCl; V_i = 70 μl; l = 90 cm; and q = 2.2 ml min^{-1}.

Table 1
Effects of various foreign species on the determination of 2×10^{-4} M penicillamine or tiopronin

Foreign species	Maximum tolerated molar ratio [species]/[drugs]
NO_3^-, SO_4^{2-}, glycine	200*
CO_3^{2-}, $C_2O_4^{2-}$, lactose, caffeine, saccharin, tartrate, citrate, alanine, valine, leucine, isoleucine, proline, threonine	100
Glucose, fructose, maltose, saccharose, phenylalanine, arginine, asparagine, histidine, salicylic acid, glutamine, lysine, aspartic acid, glutamic acid, urea	50
Starch,† hippuric acid, gelatin,† tyrosine, tryptophan, uric acid	25
Cystine, D-penicillamine disulphide	0.5
Methionine, cysteine	0.1

*Maximum molar ratio tested.
†Mass ratio.

10^{-3} M $PdCl_2$ in 1 M HCl for penicillamine and 5.0×10^{-4} M $PdCl_2$ in 0.25 M HCl for tiopronin) linear calibration graphs between 1.0×10^{-5} and 7.0×10^{-4} M for penicillamine and 1.0×10^{-5} and 6.0×10^{-4} M for tiopronin were obtained. The regression equations were: $h = 267.4 \times 10^3$ [penicillamine] + 2.26; and $h' = 270.3 \times 10^3$ [tiopronin] + 2.94; where h is the peak height in mm and drug concentrations [drug] are expressed in M; the correlation coefficients were of 0.9997 and 0.9990, respectively. The relative standard deviations for 10 determinations of 1.7×10^{-4} M penicillamine or 5×10^{-4} M tiopronin were ±0.8 and ±0.3%, respectively.

Study of possible sources of interference

The effects of foreign species on both compounds were studied. The results for the determination of 2.0×10^{-4} M penicillamine or tiopronin are listed in Table 1. Since the aim of this work is the determination of these compounds in pharmaceutical preparations, the effects of common excipients was carefully considered. The tolerance limit was taken as the concentration causing an error of not more than ±3% in the determination of each of the drugs. As can be seen, the proposed methods are sufficiently selective.

Applications

The two proposed FIA methods were applied to the determination of penicillamine or tiopronin in various pharmaceutical preparations. The results obtained and the labelled contents are summarized in Tables 2 and 3. There are no significant differences between the labelled contents and those obtained by the two proposed FIA methods for both drugs. The accuracy of the methods was checked by

Table 2
Determination of penicillamine in pharmaceutical preparations

	Penicillamine			
	Content mg per capsule/tablet			
Trade name	Found* FIA method	Nominal value	Added mg per capsule/tablet	Recovery (%)
†Cupripen capsules	253.0 ± 1.5	250	166.6	101.2
			374.9	98.7
			499.9	99.3
			749.8	101.1
†Sufortanon tablets	254.0 ± 1.2	250	100.4	101.1
			150.3	99.5
			298.4	100.8
			447.6	98.7

*Mean of five determinations ±SD.
†Composition of samples. Cupripen (Lab. Rubió, S.A.): penicillamine 250 mg and excipients per capsule. Sufortanon (Lab. Sarget): penicillamine 250 mg and excipients per tablet.

Table 3
Determination of tiopronin in pharmaceutical preparations

	Tiopronin			
	Content mg per tablet			
Trade name	Found* FIA method	Nominal value	Added mg per tablet	Recovery (%)
†Sutilan tablets	101.04 ± 1.4	100	84.2	101.1
			112.3	100.2
			168.4	99.7
			505.3	99.5
Hepadigest tablets	99.89 ± 0.44	100	49.5	101.3
			65.9	100.5
			82.6	99.3
			109.8	98.7

*Mean of five determination ±SD.
†Composition of samples. Sutilan (Lab. Cusi, S.A.): tiopronin 100 mg and excipients per tablet. Hepadigest (Lab. J. Uriach & Cia, S.A.): tiopronin 100 mg, metoclopramide hydrochloride 10 mg, cyclobutyrol calcium 100 mg, procaine base 100 mg, and excipients per tablet.

carrying out recovery studies. When known amounts of the drugs were added to commercial formulations (capsules or tablets) containing penicillamine or tiopronin, quantitative recoveries of 99.0–101% were obtained (Tables 2 and 3).

Conclusions

The proposed FIA methods for the determination of penicillamine or tiopronin showed good accuracy and reproducibility and were more sensitive than most of the FIA methods reported for these compounds. The methods proposed in this paper are fast, simple and applicable over a wide concentration range. These methods can be used as stability-indicating assays, since there is no interference from excipients that might be found in commercial preparations.

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