Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 281–284
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

In situ generation of Co(II) by use of a solid-phase reactor in an FIA assembly for the spectrophotometric determination of penicillamine

B. Gómez-Taylor Corominas [a], Julia Pferzschner [a], M. Catalá Icardo [b,1], L. Lahuerta Zamora [a], J. Martínez Calatayud [b,*]

[a] *Departamento de Química, Bioquímica y Biología Molecular, Universidad Cardenal Herrera CEU, Moncada, Valencia, Spain*
[b] *Departamento de Química Analítica, Universidad de Valencia, Valencia, Spain*

Received 24 November 2004; received in revised form 23 February 2005; accepted 25 February 2005
Available online 3 June 2005

Abstract

A flow injection analysis (FIA) manifold for the determination of penicillamine in pharmaceutical preparations is proposed. The manifold includes a solid-phase reactor for the in situ production of the derivatizing reagent, Co(II) ion, which forms a coloured complex with penicillamine in an alkaline medium. The reactor is prepared by natural immobilization of cobalt carbonate on a polymer matrix, which endows it with a high mechanical and microbiological stability. The cobalt released by passage of a 5×10^{-4} mol l^{-1} sulphuric acid stream at a flow-rate of 2.3 ml min^{-1} is merged with a volume of 314 μl of sample containing penicillamine in ammonium–ammonia buffer at pH 9.5 to measure the absorbance at 360 nm. Beer's law is obeyed over the penicillamine concentration range 5–60 mg l^{-1}. The limit of detection (LOD) of the method is 1 mg l^{-1} and its throughput 70 samples h^{-1}.

Keywords: Penicillamine; Flow injection analysis, FIA; Solid-phase reactor; Pharmaceuticals; Cobalt

1. Introduction

Penicillamine (3,3-dimethylcysteine) is the main product of the decomposition of penicillin antibiotics [1]. It is typically used to treat Wilson's disease—which results from the presence of exceeding high concentrations of copper in the body, rheumatoid arthritis, cystinuria and lead poisoning.

The presence of a thiol, amino and carboxyl group in this compound provides a number of ways of interacting with organic [2–4] and inorganic [5–9] species or both [10,11], in a variety of complex formation and redox reactions that yield some spectrophotometrically active product.

There have been many attempts at automating the determination of penicillamine in pharmaceutical preparations by use of non-separation flow techniques (largely FIA, but also SIA [5]) in conjunction with spectrophotometric [5,7,8], fluorimetric [12], electrochemical [13–15] and chemiluminescence-based [16–18] detection.

Spetrophotometric flow injection methods for the determination of penicillamine rely on the formation of coloured chelates with various metal cations (see Table 1). This approach was also used in this work; however, the metal cation employed as reagent was obtained in situ by using a solid-phase reactor.

The advantages of inserting a solid-phase reactor into a continuous-flow manifold were discussed in previous papers [19–22]. Worth special note is the ability to simplify manifolds, save reagents, implement cleaner chemistries, raise the sensitivity and throughput, reduce labour and, frequently, decrease the release of excess reagents as waste. Solid-phase reactors have proved economical, stable, reproducible long-life supplies of reagents. This work confirms the advantages of using solid reagents in flow systems. The reactor used [19] contained cobalt carbonate immobilized on a polymer

* Corresponding author. Tel.: +34 96 354 4062; fax: +34 96 354 4062.
E-mail address: jose.martinez@uv.es (J. Martínez Calatayud).
URL: http://www.uv.es/~martinej/, http://www.uv.es/~martinej/FlowAnalysis.
[1] Present address: Departamento de Química, Universidad Politécnica de Valencia, Valencia, Spain.

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.02.044

Table 1
Comparing the proposed flow procedure vs. other spectrophotometric procedures for penicillamine determination

References	Flow modality	Derivatizing reagent (λ)	Linear range (mg l^{-1})	Sample throughput (h^{-1})
[5]	Sequential injection	Fe(III) complex in HCl (600 nm)	25–300	48
[7]	FIA	Pd(II) complex in HCl (400 nm)	1.5–104	–
[8]	FIA	Co(II) complex in ammonium acetate (360 nm)	15–300 (peak-height) 7.5–14900 (peak-base-width)	150
Proposed FIA procedure	FIA	Co(II) complex from a solid-phase reactor in buffer at pH 9.5 (360 nm)	5–60	70

matrix. Cobalt(II) ion was released from the reactor by continuously circulating a dilute solution of sulphuric acid, and the complex formed between the cation and penicillamine in an alkaline medium was monitored at 360 nm.

2. Experimental

2.1. Reagents

All reagents were of analytical grade unless stated otherwise. Aqueous solutions were prepared in pure (reverse osmosis) and de-ionised water (system Sybron/Barnstead Nanopure II provided with a filter 0.2 μm pore size). Penicillamine was obtained from Fluka. Other reagents were: H_2SO_4, $CoCO_3{\cdot}6H_2O$, NaOH, NH_4Cl, $Na_2B_4O_7$, Na_3PO_4, NH_3, D-glucose and lactose 1-hydrate all from Panreac, saccharin, starch, magnesium stearate and sucrose all from Guinama and sucrose from Boehringer. Polyester resin Al-100-A from Reposa and catalyst (ethyl methyl ketone peroxide) from Azko.

2.2. Apparatus

The flow manifold, depicted in Fig. 1, comprised a Rheodyne 5041 injection valve and a peristaltic pump Minipuls 2 (from Gilson). All tubing (Omnifit) was 0.5 mm internal diameter, except for the solid-phase reactor, which was 1.5 mm. Two different spectrophotometric detectors were used: a diode array spectrophotometer (Model 8452 from Hewlett–Packard) when spectra were required and a photometer (Model CE292 from Cecil Instruments), both provided with 18 μl inner volume a flow cell (Hellma).

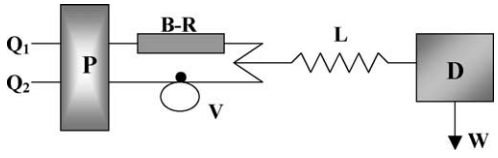

Fig. 1. Flow injection system proposed for the penicillamine determination. V, injection valve, 314 μl of sample solution in NH_4^+/NH_3 buffer 0.02 mol l^{-1} at pH 9.5; P, peristaltic pump; Q_1, 5×10^{-4} mol l^{-1} sulphuric acid at 2.3 ml min^{-1}; Q_2, de-ionised water at 2.4 ml min^{-1}; B-R, solid-phase reactor; L, coil reactor of 100 cm; W, waste; D, spectrophotometer detector working at 360 nm.

2.3. Procedures

2.3.1. Preparation of the solid-phase reactor

The reactor was prepared according to the procedure formerly published [19]. The filling particles were prepared as follows: 30 g of $CoCO_3{\cdot}6H_2O$ was mixed with 30 g of the polymer resin solution Al-100 (from Reposa) by manual stirring, and then 1.2 g of ethyl methyl ketone was added and stirred until polymer became too rigid; final ratio $CoCO_3$:resin was 1:1. After drying for 2–3 h at room temperature, the solid was broken with a hammer and ground in a coffee grinder. Particle size 100–200 μm was selected by sieving, washed and dried at 80 °C, sieved again and stored. The solid-phase reactor was prepared by filling a PTFE tubing (14 cm × 1.5 mm i.d.). The empirical study on the reactor stability revealed constant analytical outputs at least during 6.5 h, which was equivalent to 450 determinations.

2.3.2. Proposed flow injection method for penicillamine determination

The flow injection assembly is depicted in Fig. 1. Three hundred and fourteen microliters of sample (a solution of penicillamine in NH_4^+/NH_3 buffer 0.02 mol l^{-1} at pH 9.5) was inserted into a stream of water at 2.4 ml min^{-1} (Q_2) and merges with the Co(II) solution from the solid-phase reactor, liberated by a solution 5×10^{-4} mol l^{-1} of sulphuric acid flowing at 2.3 ml min^{-1} (Q_1). The resulting mixture is pumped to the detector flow cell trough a 100 cm reactor and the absorbance at 360 nm is recorded.

3. Results and discussion

3.1. Preliminary tests

In an alkaline medium, the Co(II) supply by the solid-phase reactor forms a Co(II):cysteine complex of 1:2 stoichiometry that can be spectrophotometrically determined at 360 nm [19].

Penicillamine is a cysteine derivative the only difference from which is the presence of two methyl groups instead of two protons at C_3. Because cysteine and cobalt interact via the sulphur and nitrogen atoms in the amino group penicillamine can be expected to form similar compounds, as confirmed in previous work [8], by visual inspection and from spectra

recorded in the visible region. Tests revealed that the reaction between Co(II) and penicillamine only takes place above pH 6 and also like cysteine, it yields a coloured compound.

Preliminary continuous-flow tests were performed by using the previously described system optimized for the determination of cysteine [19], where the sample solution, prepared in 5×10^{-4} M sulphuric acid, goes through the $CoCO_3$ solid-phase reactor. Under these conditions, the signal for penicillamine was found to decrease upon repeated insertion of sample volumes; this suggests that the analyte somehow deactivated the solid-phase reactor with time—which was not the case with cysteine.

3.2. Selection of the FIA assembly

The FIA manifold used to avoid passage of the analyte through the reactor is shown in Fig. 1. Cobalt(II) was released from the reactor by continuously passing through it 5×10^{-4} mol l^{-1} H_2SO_4 stream (Q_1) at 2.3 ml min^{-1}. No appreciable change of the reactor stability was observed after passing a sulphuric acid solution at a concentration of 0.025 mol l^{-1} (i.e. 50 times higher than the experimental level) at a flow-rate of 1.4 ml min^{-1} for more than 1 h. Channel Q_2 in the manifold was used to circulate a de-ionised water carrier at 1.8 ml min^{-1} into which a volume of 182 μl of penicillamine solution in 0.04 mol l^{-1} borax buffer at pH 9.5 was inserted—using the buffer solution as carrier resulted in no improvement. The preselected length for the mixing reactor in this preliminary work was 117.0 cm.

3.3. Optimization of the FIA system

Once the reactor was checked to be stable, the new FIA system was optimized by examining the influence of chemical and FIA variables.

Two different buffers ($B_4O_7^{2-}$/OH^- and NH_4^+/NH_3^+) at variable concentrations (0.01–0.15 mol l^{-1}) and pH values (8.5–10.4) were studied and a 0.02 mol l^{-1} NH_4^+/NH_3 buffer at pH 9.5 was found to be provide the best results. The buffer concentration was scarcely influential.

The hydrodynamic flow variables studied included the inserted volume (V), mixing reactor length (L) and penicillamine carrier flow-rate (Q_2). Fig. 2 shows the variation of each variable over its studied range. The values adopted as optimal were an inserted volume of 134 μl, a mixing reactor length of 100 cm and a carrier flow-rate of 2.4 ml min^{-1}. The reported conditions established (flow-rate through the mixing coil 4.7 ml min^{-1}) the best relationship reaction rate-sample dispersion in 2.5 s, which means a non-slow reaction rate.

3.4. Analytical applications

The relationship between the analytical signal and concentration was studied over the range 5–150 mg l^{-1} and found to be linear from 5 to 60 mg l^{-1}. The corresponding fitted

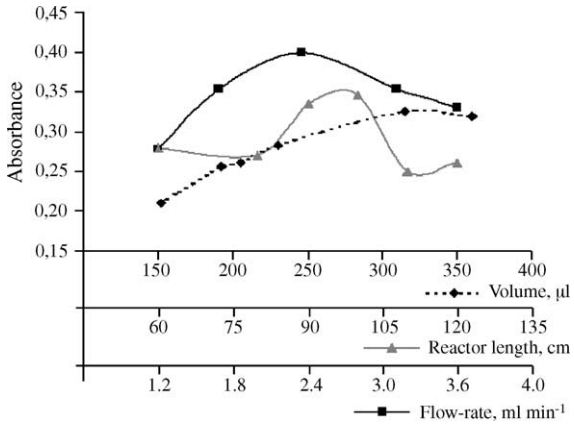

Fig. 2. Influence of hydrodynamic parameters (inserted volume, reaction coil length and carrier flow-rate) on the analytical outputs.

equation ($n = 4$) was

$$A = 0.0064 + 0.0055C \quad (r = 0.996)$$

where A is the absorbance at 360 nm and C is the penicillamine concentration in mg l^{-1}.

The limit of detection was experimentally determined by inserting penicillamine solutions of decreasing concentration and taken to be that providing a signal coinciding with the baseline width plus three times its standard deviation. The value thus found was 1 mg l^{-1}.

The repeatability of the system was determined from 30 consecutive insertions of a 40 mg l^{-1} solution of penicillin; the relative standard deviation (R.S.D.) thus obtained was 1.3%. The calculated throughput for this experimental series was 70 samples h^{-1}.

The potential interferents studied included various excipients present in commercially available penicillamine formulations. Solutions containing the analyte at a 25 mg l^{-1} concentration and the interferents at levels above those typically found in the formulations were used for this purpose. A compound was deemed an interferent when the error in the signal for a 25 mg l^{-1} standard of penicillamine exceeded 3%, in which case the concentration of the compound concerned was lowered until no interference was observed. Table 2 shows the results obtained.

Table 2
Influence of foreign compounds

Interference	Concentration (mg l^{-1})	E_r (%)
Starch[a]	108	+2.7
Saccharin	1000	−2.6
Glucose	1000	−2.0
Sodium phosphate	1000	−1.7
Magnesium stearate[a]	20	−2.1
Lactose	1000	−2.2
Sucrose	1000	+1.7

[a] These “solutions” were prepared by adding 0.027 and 0.010 g of starch and magnesium stearate, respectively, to 200 ml of water and leveling to 250 after heating.

Finally, the proposed method was used to determine penicillamine in the commercial pharmaceutical preparation Cupripen 50 from Laboratorios Rubio. An appropriate number of tablets was weighed and ground in an agate mortar. An aliquot of the ground solid was weighed, dissolved and made to an appropriate volume with the same buffer as for the standards, the solution being diluted as required to obtain a penicillamine concentration within the linear concentration range for the proposed method. The penicillamine concentration thus obtained was (52.6 ± 1.5) ppm ($n = 3$); the manufacturer's stated nominal concentration was 50 ppm.

4. Conclusions

The use of a solid-phase reactor consisting of cobalt carbonate immobilized on a polymer matrix is an effective alternative to dissolved cations as reflected in the sensitivity and throughput data of Table 1, which compares the analytical figures of merit of selected reported non-separation continuous-flow methods for the spectrophotometric determination of penicillamine.

The proposed solid-phase reactor provides a stable, reproducible supply of Co(II) for the determination of penicillamine by formation of a coloured complex in an alkaline medium and its spectrophotometric detection at 360 nm.

The use of immobilized solid reagents instead of frequently unstable solutions results in time and labour savings and often avoids the release of excess reagents as waste.

References

[1] Analytical Profiles of Drugs Substances, vol. 10, Ed. Board, 1981.
[2] Y. Fujita, I. Mori, T. Yamaguchi, Anal. Sci. 18 (2002) 981–985.
[3] A.A. Al-Majed, J. Pharm. Biomed. Anal. 21 (1999) 827–833.
[4] V. Amarnath, K. Amarnath, Talanta 56 (2002) 745–751.
[5] F.E.O. Suliman, H.A.J. Al-Lawati, S.M.Z. Al-Kindy, I.E.M. Nour, S.B.A. Salama, Talanta 61 (2003) 221–231.
[6] B. Stypinski Mis, G. Anderegg, Anal. Chim. Acta 406 (2000) 325–332.
[7] M.S. García, C. Sánchez Pedreño, M.I. Albero, V. Rodenas, J. Pharm. Biomed. Anal. 11 (1993) 633–638.
[8] P. Viñas, J.A. Sánchez Prieto, M. Hernández Cordoba, Microchem. J. 41 (1990) 2–9.
[9] A. Besada, Anal. Lett. 21 (1988) 435–446.
[10] V.K. Gupta, I. Ali, Talanta 46 (1998) 197–201.
[11] P.B. Issopoulos, S.E. Salta, Farmaco 52 (1997) 113–118.
[12] T. Pérez Ruiz, C. Martínez Lozano, V. Tomas, C. Sidrach de Cardona, J. Pharm. Biomed. Anal. 15 (1996) 33–38.
[13] N. Wangfuengkanagul, O. Chailapakul, Talanta 58 (2002) 1213–1219.
[14] M. Catalá Icardo, O. Armenta Estrela, M. Sajewicz, J.V. García Mateo, J. Martínez Calatayud, Anal. Chim. Acta 438 (2001) 281–289.
[15] G. Favaro, M. Fiorani, Anal. Chim. Acta 332 (1996) 249–255.
[16] Z.D. Zhang, W.R.G. Baeyens, X.R. Zhang, G. Van der Weken, Analyst 121 (1996) 1569–1572.
[17] Z.D. Zhang, W.R.G. Baeyens, X.R. Zhang, G. Van der Weken, Biomed. Chromatogr. 9 (1995) 287–288.
[18] I. López García, P. Viñas, J.A. Martínez Gil, Fresenius J. Anal. Chem. 345 (1993) 723–726.
[19] M. Catalá Icardo, L. Lahuerta Zamora, J. Martínez Calatayud, Analyst 123 (1998) 1685–1689.
[20] J. Martínez Calatayud, Flow injection Analysis of Pharmaceuticals. Automation in the Laboratory, Taylor and Francis, Cambridge, 1996.
[21] J.V. García Mateo, J. Martínez Calatayud, Anal. Chim. Acta 274 (1993) 275–281.
[22] J. Martínez Calatayud, L. Lahuerta Zamora, L. Anal. Chim. Acta 300 (1995) 143–148.