Journal of Chromatography, 318 (1985) 404–407
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,309

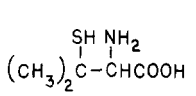
Note

Determination of penicillamine in encapsulated formulations by high-performance liquid chromatography

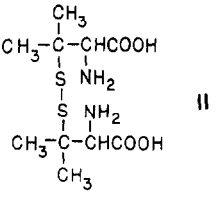
S. BIFFAR*, V. GREELY and D. TIBBETTS
Merck Sharp and Dohme, West Point, PA 19486 (U.S.A.)
(First received April 24th, 1984; revised manuscript received October 16th, 1984)

Penicillamine (β,β-dimethylcysteine, I) is a thiol chelating agent used for the removal of excess copper in patients with Wilson's disease. The compound has also been used for the treatment of severe cases of rheumatoid arthritis. The mechanism of action of penicillamine in rheumatoid arthritis is unknown.

The analysis of penicillamine by radioimmunoassay[1,2], gas chromatography[3], and colorimetry[4,5] has been reported in the literature. The USP determination of penicillamine consists of a colorimetric titration with mercuric acetate[6]. These methods either require complex sample preparation or are not specific for penicillamine and its major degradation product penicillamine disulfide (II).



I



II

Several high-performance liquid chromatography (HPLC) assays have been reported recently incorporating various detection methods. These include electrochemical detection[7–9], fluorescence derivatization[10,11] and post-column reaction with Ellman's regent[12]. We have developed a reversed-phase HPLC procedure with UV detection which is suitable for the routine analysis of penicillamine in encapsulated products. This method is simple, specific, and sensitive to 10^{-5} *M* levels of penicillamine.

EXPERIMENTAL

Reagents and standards

USP reference standard penicillamine was used in the standard solution. Disodium EDTA, sodium phosphate monobasic, hexanesulfonic acid sodium salt, and phosphoric acid (85%) (Fisher Scientific, Fair Lawn, NJ, U.S.A.) were used as received. Deionized-distilled water was used throughout the procedure.

A penicillamine standard was prepared by dissolving penicillamine USP ref-

0021-9673/85/$03.30

erence standard in 0.1% disodium EDTA (Na_2H_2EDTA) to obtain a concentration of 1.25 mg/ml for the analysis of the encapsulated products.

Chromatography

The HPLC system used consisted of a Varian Model 5060 liquid chromatograph equipped with a 20-μl loop-injector, a variable-wavelength detector (Spectromonitor III, Model 1204, LDC Instrument Co.) set at 210 nm, and an ASI 30 cm × 4.0 mm I.D. 10-μm ODS column (ASI, Santa Clara, CA, U.S.A.) operated at ambient temperature. The mobile phase was 0.05 *M* sodium phosphate monobasic, 0.001 *M* hexanesulfonic acid sodium salt (HSA) adjusted to pH 3.0 with phosphoric acid. The flow-rate was 1.7 ml/min.

Sample preparation

The contents of ten penicillamine capsules were dissolved in 0.1% disodium EDTA to give a final concentration of 1.25 mg/ml. Samples were filtered through a 0.45-μm membrane filter (HAWP 01300, Millipore).

RESULTS AND DISCUSSION

This HPLC procedure separates penicillamine from the penicillamine disulfide (Fig. 1). Penicillamine disulfide is formed by the oxidative coupling of two penicillamine molecules.

The resolution of penicillamine and the disulfide is affected by the concentration of the HSA in the mobile phase. The relationship of the HSA content in the mobile phase to the retention time of the penicillamine and penicillamine disulfide is shown in Table I. As the concentration of the HSA is increased, the disulfide is retained to a greater extent than the penicillamine.

The stability of penicillamine in solution was studied to find stable conditions for samples and standards. We confirmed the results of Rabenstein and Saetre[7] that penicillamine was most stable in 0.1% disodium EDTA. Disodium EDTA stabilizes the penicillamine solution by complexing trace metals which catalyze thiol oxidation[13].

TABLE I

RELATIONSHIP OF THE RETENTION TIME TO THE HEXANESULFONIC ACID SODIUM SALT CONCENTRATION IN THE MOBILE PHASE

The mobile phase consisted of aqueous 0.05 *M* phosphate buffer, pH 3.0, at a flow-rate of 1.7 ml/min; a 30 × 0.40 cm I.D. octyldecyl-bonded silica column was used.

HSA concentration (M)	*Retention time (min)*	
	Penicillamine	*Penicillamine disulfide*
$5 \cdot 10^{-4}$	3.80	5.60
$1 \cdot 10^{-3}$	4.45	7.15
$2 \cdot 10^{-3}$	5.15	9.50
$4 \cdot 10^{-3}$	6.00	13.45

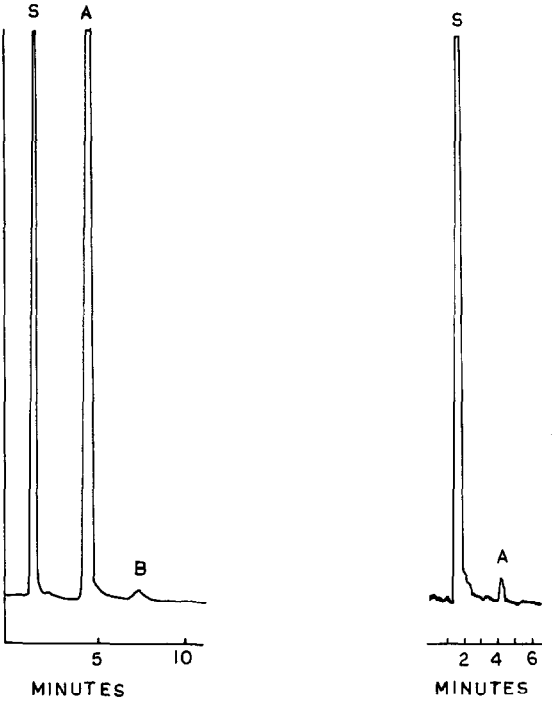


Fig. 1. Reversed-phase ion-pair separation of penicillamine from penicillamine disulfide. Peaks: S = solvent; A = penicillamine; B = penicillamine disulfide.

Fig. 2. Reversed-phase ion-pair HPLC determination of 40 ng of penicillamine in a solution of EDTA (1 g/l). Peaks: S = solvent; A = penicillamine.

TABLE II

COMPARISON OF HPLC AND USP XX METHOD ASSAY RESULTS FOR PENICILLAMINE CAPSULES

Capsule strength (mg)	*Age (months)*	*HPLC*				*USP XX**	
		Penicillamine		*Penicillamine disulfide*		*Penicillamine*	
		mg/caps.	*Claim (%)*	*mg/caps.*	*Penicillamine equivalent (%)*	*mg/caps.*	*Claim (%)*
125	45	128.5	102.8	0.97	1.55	126.8	101.4
	42	121.0	96.8	1.83	2.93	118.5	94.8
	40	127.5	102.0	1.31	2.09	127.0	101.6
	38	125.5	100.4	1.36	2.18	125.3	100.2
	34	120.6	96.5	1.19	1.90	121.6	97.3
250	45	243.5	97.4	1.95	1.56	243.0	97.2
	44	250.0	100.4	2.09	1.67	250.3	100.1
	16	242.5	97.0	2.76	2.21	242.8	97.1
	23	245.0	98.0	1.40	1.12	244.8	97.9
	7	263.3	105.3	2.39	1.91	263.3	105.3

* Titration with mercuric acetate using diphenylcarbazone as an indicator[6].

The reproducibility of the chromatographic method was shown by chromatographing 10 injections of a standard solution containing 1.25 mg penicillamine/ml. The relative standard deviation of the peak areas was ±0.16%.

The percent recovery of penicillamine from a spiked placebo formulation averaged 100.2 ± 0.1 for 6 determinations. The standard was step-wise diluted to determine the limit of detection. A 20-μl injection of a $2 \cdot 10^{-3}$ mg/ml standard was determined to be the lower limit of detection (Fig. 2). This level represents 40 ng of penicillamine. UV detection, although not as sensitive as electrochemical and fluorescence detection, provides a reliable and simplified method of detection for the analysis of penicillamine products.

Five lots each of 125-mg and 250-mg capsules were assayed by this method. The results were compared with the official USP procedure[6]. Table II shows that the levels of penicillamine varied between 96.5 and 105.3% of the label claim amount using the HPLC procedure. The results are comparable for the two methods. The HPLC procedure has the advantage of separating and measuring the amount of disulfide impurity present in each lot of capsules. The results in Table II show that less than 3% of the penicillamine in each lot is in the form of the disulfide.

The HPLC assay procedure is rapid, quantitative, sensitive, and represents a more convenient assay method than that in the current USP. This method is suitable for the quality control of solid dosage forms of penicillamine.

REFERENCES

1 E. S. K. Assem, *Curr. Med. Res. Opin.*, 2 (1974) 568–572.
2 E. S. K. Assem and M. R. Vickers, *Post-Grad. Med J.*, 50 (1974) 10–14.
3 E. Jelleum, V. A. Bacon, W. Patton, W. Pereira, Jr. and B. Halpern, *Anal. Biochem.*, 31 (1969) 339–347.
4 P. R. Pal, *J. Biol. Chem.*, 243 (1959) 618–619.
5 J. Mann and P. O. Mitchell, *J. Pharm. Pharmacol.*, 31 (1979) 420–421.
6 *The United States Pharmacopeia*, Mack Publishing Co., Easton, PA, 20th rev., 1980, p. 591.
7 D. L. Rabenstein and R. Saetre, *Anal. Chem.*, 50 (1978) 276–280.
8 R. F. Bergstrom, D. R. Kay and J. G. Wagner, *J. Chromatogr.*, 222 (1981) 445–452.
9 I. C. Shaw, A. E. M. McLean and C. H. Boult, *J. Chromatogr.*, 275 (1983) 206–210.
10 E. P. Lankmayr, K. W. Budna, K. Müller, F. Nachtmann and F. Rainer, *J. Chromatogr.*, 222 (1981) 249–255.
11 J. O. Miners, I. Fearnley, K. J. Smith, D. J. Birkett, P. M. Brooks and M. W. Whitehouse, *J. Chromatogr.*, 275 (1983) 89–96.
12 D. Beales, R. Finch, A. E. M. McLean, M. Smith and I. D. Wilson, *J. Chromatogr.*, 226 (1981) 498–503.
13 P. C. Jocelyn, *Biochemistry of the SH Group*, Academic Press, New York, NY, 1972, pp. 94–110.