Journal of Chromatography, 549 (1991) 410–415
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 337

Short Communication

High-performance liquid chromatographic method for the determination of cloxacillin in commercial preparations and for stability studies

MEI-CHICH HSU* and MEI-CHAI CHENG

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2, Kuen-Yang St., Nankang, Taipei 11513 (Taiwan)

(First received December 20th, 1990; revised manuscript received March 26th, 1991)

ABSTRACT

A reversed-phase column liquid chromatographic method was developed for the assay of cloxacillin and its preparations. The linear calibration range was 0.2–3.0 mg/ml ($r = 0.9998$) and recoveries were generally greater than 99%. The relative standard deviation was 0.13% ($n = 10$). The high-performance liquid chromatographic assay results were compared with those obtained from a microbiological assay for bulk drug substance, capsule, injection and syrup formulations containing cloxacillin and degraded cloxacillin. At the 99% confidence level, no significant inter-method differences were noted for the paired results. Commercial formulations were also analysed and the results obtained by the proposed method closely agreed with those found by the microbiological method. The results indicated that the proposed method is a suitable substitute for the microbiological method for assays and stability studies of cloxacillin preparations.

INTRODUCTION

The US *Code of Federal Regulations* [1] described two official methods for potency assay of cloxacillin: a microbiological method and iodimetric titration. The regulations state that the results obtained from the microbiological method shall be conclusive. The present official assay method of *Minimum Requirements for Antibiotic Products of Japan* [2] for the analysis of potency of cloxacillin in bulk drug substance and its preparations is a microbiological method.

However, there has recently been a move to replace expensive microbiological assays by chemical assays, *e.g.*, high-performance liquid chromatography (HPLC). Several HPLC methods for the determination of cloxacillin in biological fluids have been reported [3–11]. Fewer methods have been reported for separating and identifying cloxacillin and the derivatives of penicillin [12–14] and for the determination of cloxacillin in pharmaceutical samples [15].

0021-9673/91/$03.50

In order to establish whether an HPLC method would be acceptable, it is important to determine whether it is robust enough for assaying samples kept under extreme conditions. Degradation in the sample should be equally reflected by microbiological and HPLC assays. This paper describes a comparison of a proposed HPLC method with a microbiological assay for the determination of cloxacillin in commercial formulations. Further, cloxacillin was kept at elevated temperatures as part of an accelerated degradation experiment and assayed by microbiological and HPLC methods.

EXPERIMENTAL

Instruments

A Waters Model 600E liquid chromatographic pump (Waters Chromatography Division, Millipore, Milford, MA, USA), a Waters Model 490E UV detector and a Waters Model 745 Data Module were employed during the study. The mobile phase was pumped through a reversed-phase column (μBondapak C_{18}, 30 cm × 3.9 mm I.D., 10 μm; Waters P/N 27324) with an isocratic flow-rate of 1.5 ml/min. The detector was set at 254 nm. Chromatography was performed at room temperature. Injections of 10 μl were made of all solutions to be analysed.

Mobile phase

The mobile phase was methanol–4% acetic acid (60:40, v/v). The mobile phase was filtered (0.45-μm Millipore filter) and degassed with an ultrasonic bath prior to use.

Standard solutions

Internal standard dimethyl phthalate (3 g) was dissolved in 100 ml of acetonitrile–water (1:1). To an accurately weighed amount of cloxacillin sodium standard (National Laboratories of Foods and Drugs, Taiwan), equivalent to 50 mg potency of cloxacillin, was added 0.5 ml of internal standard stock solution and the volume was made up to 50.0 ml with distilled water.

Sample preparations

To an accurately weighed amount of bulk drugs, homogeneous capsule contents, injection or syrup formulations, equivalent to 50 mg potency of cloxacillin was added 0.5 ml of internal standard stock solution, and the volume was made up to 50.0 ml with distilled water.

Solution for linearity response

Seven concentrations of cloxacillin sodium, which ranged from 0.2 to 3.0 mg/ml, were prepared. Each concentration was chromatographed six times.

Solution for recovery studies

To an accurately wieghed 50 mg potency of sample composites of commercial preparations were added different amounts of cloxacillin standard and 0.5 ml of internal standard stock solution. Each solution was made up to 50.0 ml with distilled water and was chromatographed in triplicate.

Microbiological assay procedure

Bacillus subtilis (Culture Collection and Research Center, Taiwan) was used in the microbiological assay. According to the cup plate method, standards and test drugs were diluted to 1.0 mg/ml (potency) concentrated solution with distilled water and then diluted to 16.0 and 4.0 μg/ml with 1% phosphate buffer solution (pH 6.0) on the day of analysis. Five Petri dishes of 9.0 cm I.D. were used for each sample. After incubation for 16–18 h, the zone diameter was measured by a zone analyser (Model ZA-F; Toyo, Tokyo, Japan).

RESULTS AND DISCUSSION

The linearity of the peak-area ration (cloxacillin *vs.* internal standard) was verified by injection of seven solutions containing cloxacillin in the concentration range 0.2–3.0 mg/ml. A straight line with a correlation coefficient of 0.9998 ($y = 0.0379 + 0.1483x$) was obtained.

The reproducibility (C.V.) of the proposed method, on the basis of peak-area ratios for ten replicate injections was 0.13%. The standard deviation was 0.19%.

The results of standard addition recovery studies of cloxacillin from sample composites of commercial preparations are shown in Table I. The average recovery was greater than 99%. These data indicate that the proposed HPCL method is relatively unaffected by the sample matrix.

Typical chromatograms of cloxacillin commercial dosage forms are shown in Fig. 1. The retention time was about 3.7 min for the internal standard and 5 min for cloxacillin. Excipients from commercial formulations did not interfere.

When samples of capsule, injection and syrup formulations were heat degraded, the resulting mixtures yielded chromatograms containing additional peaks, none of which interfered with the interpretation and measurement of the chromatographic peaks for cloxacillin and dimethyl phthalate, as shown in Fig. 1. In addition, a decrease in peak height (and/or peak area) with increase in temperature and time can be observed.

TABLE I

RECOVERY OF CLOXACILLIN FROM VARIOUS COMMERCIAL COMPOSITES

Formulation	Added (mg)	Found (mg)	Recovery (%)	Average recovery (%)
Capsule	11.1	11.18	100.7	99.83
	16.7	16.58	99.3	
	22.2	22.08	99.5	
Injection	11.8	11.73	99.4	99.57
	16.7	16.51	98.9	
	22.3	22.39	100.4	
Syrup	11.7	11.81	100.9	100.37
	16.9	17.20	101.8	
	22.2	21.04	98.4	

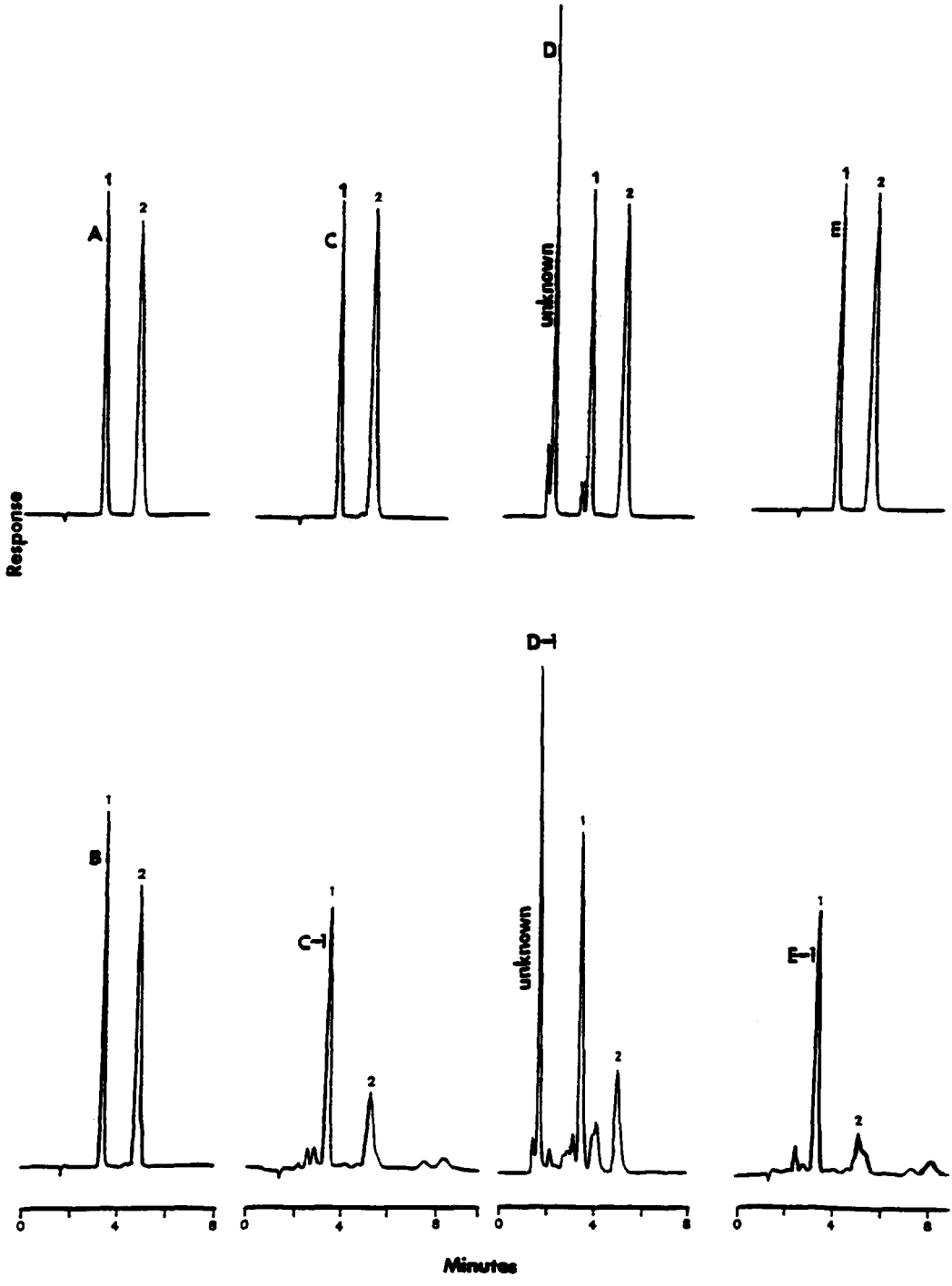

Fig. 1. Chromatograms of cloxacillin preparations: (A) house standard; (B) bulk drug substance; (C) 250-mg capsule; (C-1) degraded 250-mg capsule; (D) 125 ml per 5 ml syrup; (D-1) degraded 125 mg per 5 ml syrup; (E) 250 mg per vial injection; (E-1) degraded 250 mg per vial injection. Peaks: 1 = dimethyl phthalate; 2 = cloxacillin (10 μg).

TABLE II

COMPARISON OF MICROBIOLOGICAL AND HPLC ASSAYS FOR CLOXACILLIN

Sample	Potency[a]	
	Microbiological method[b]	HPLC[c]
Bulk drug		
House standard	899.7	899.7
USP standard	924.8	920.3
Brand A: 1	849.2	855.9
2	917.0	893.0
3	900.2	892.4
Brand B: 1	887.2	891.6
2	927.8	920.6
3	925.0	914.6
Dosage form, declared		
Brand A: 1 250 mg/cap.	104.1	103.2
2 250 mg/cap.	102.5	102.2
Brand C: 250 mg/cap.	101.1	102.2
Brand D: 1 250 mg/cap.	100.2	102.8
2 250 mg/vial	105.0	105.7

[a] The potency was determined as μg/mg for bulk drug and percentage of declared for dosage form.
[b] Average of five determinations.
[c] Average of three determinations.

TABLE III

COMPARISON OF PERCENT POTENCY OF CLOXACILLIN IN CAPSULE, INJECTION AND SYRUP FORMULATIONS AS DETERMINED BY MICROBIOLOGICAL AND HPLC METHODS

% of declared concentration

Capsule, 250 mg		Injection, 250-mg vial		Syrup, 125 mg per 5 ml	
Microbiological method	HPLC	Microbiological method	HPLC	Microbiological method	HPLC
107.4	107.3	109.9	105.0	117.2	116.4
107.1	101.3	106.1	105.9	113.7	114.7
105.9	106.3	105.8	106.3	112.8	107.4
105.7	101.5	105.5	103.3	102.2	101.8
103.5	104.4	92.7	90.9	99.4	96.7
103.2	102.1	90.1	89.2	91.5	87.9
102.8	100.8	88.5	85.2	89.7	84.0
101.8	104.1	73.6	72.3	75.2	72.0
101.3	101.0	51.6	54.2	74.3	71.5
101.1	102.3	44.0	43.9	62.1	58.5
100.8	100.1	18.0	21.1	45.9	47.4
99.9	102.4	15.7	18.2	42.0	43.5
90.1	88.2			25.9	27.6
84.8	86.1				
76.6	77.6				
38.2	43.7				
37.0	39.3				
31.4	26.6				

A number of samples of bulk drug substance and commercial preparations of three brands were analysed for cloxacillin content by HPLC. These samples were also assayed by the microbiological method. The results are shown in Table II. A *t*-test was applied to the data: analysis showed no significant difference at the 99% confidence level for any of the preparations when assayed by the microbiological or HPLC methods.

A study was initiated to ascertain the suitability of the proposed method for stability studies. Samples of capsule, injection and syrup formulations were stored in temperature-controlled cabinets (ambient or 55–150°C). Samples were taken from the cabinets periodically for microbiological and HPLC assays. The assay values, expressed as a percentage of the label claim, are given in Table III. The paired values in Table III have correlation coefficients of 0.994 for capsule, 0.999 for injection and 0.997 for syrup dosage forms, respectively. Hence no significant difference in the assay values obtained by the two analytical methods was found for degraded or non-degraded samples.

This study demonstrates the applicability of the proposed HPLC method for the potency determination of cloxacillin in bulk drug and capsule, injection and syrup formulations. The method can be successfully used for routine quality control and stability assays and offers advantages in speed, simplicity and reliability.

ACKNOWLEDGEMENTS

The authors thank Bristol Industries and China Biological and Chemical Laboratories for the supply of cloxacillin sodium bulk drugs.

REFERENCES

1 *Code of Federal Regulations, Title 21, Part 440*, US Government Printing Office, Washington, DC, 1988.
2 *Minimum Requirements for Antibiotic Products of Japan*, English Version, Japan Antibiotics Research Association, Tokyo, 1986.
3 H. H. W. Thijssen, *J. Chromatogr.*, 183 (1980) 339.
4 A. Brunetta, L. Mosconi, S. Pongiluppi, U. Scagnolari and G. Zambonin, *Boll. Chim. Farm.*, 120 (1981) 335.
5 F. W. Teare, R. H. Kwan, M. Spino and S. M. MacLeod, *J. Pharm. Sci.*, 71 (1982) 938.
6 W. A. Moats, *J. Chromatogr.*, 317 (1984) 311.
7 D. M. Paton, *J. Clin. Pharmacol. Res.*, 6 (1986) 347.
8 F. Jehl, P. Birckel and H. Monteil, *J. Chromatogr.*, 413 (1987) 109.
9 M. A. Abuirjeie and M. E. Abdel Hamid, *J. Clin. Pharmacol. Ther.*, 13 (1988) 101.
10 C. T. Hung, J. K. C. Lim, A. R. Ziest and F. C. Lam, *J. Chromatogr.*, 425 (1988) 331.
11 H. S. Lee, T. Y. Yi and Y. M. Khoo, *Ther. Drug Monit.*, 10 (1988) 340.
12 G. T. Briguglio and C. A. Lau-Cam, *Assoc. Off. Anal. Chem.*, 67 (1984) 228.
13 J. Martin, R. Mendez and A. Negro, *J. Liq. Chromatogr.*, 11 (1988) 1707.
14 M. A. S. Salem and H. N. Alkaysi, *Drug Dev. Ind. Pharm.*, 13 (1987) 2771.
15 G. Lauriault, M. J. Lebelle and A. Vilim, *J. Chromatogr.*, 246 (1982) 157.