Journal of Chromatography, 246 (1982) 157–160
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,058

Note

High-performance liquid chromatographic determination of cloxacillin in pharmaceutical dosage forms

G. LAURIAULT*, M. J. LeBELLE and A. VILIM
Bureau of Drug Research, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa K1A OL2 (Canada)
(Received May 24th, 1982)

The present official assay method of the British Pharmacopoeia (B.P.)[1] for analysis of cloxacillin in solid oral dosage forms involves the colorimetric determination of penicillins by reaction in imidazole–mercury solution as described by Bundgaard[2]. The Code of Federal Regulations[3] prescribes an iodometric method or the microbiological agar diffussion assay. The results obtained from the microbiological method are to be considered conclusive.

Other chemical methods reported in the literature involve ninhydrin reaction and measurement of the extinction at 570 nm (Celletti *et al.*[4]), ammonium vanadate reaction and measurement at 750 nm (Ibrahim *et al.*[5]) or a hydrolysis in 1 *M* hydrochloric acid to a product absorbing at 345 nm (Szawlowska and Borowicz[6]).

Canadian drug quality assessment programs require reliable and timesaving methods for the analysis of the large number of samples. Due to the lack of specificity of the official methods, a reversed-phase high-performance liquid chromatographic (HPLC) method for the determination of cloxacillin sodium in pharmaceutical oral dosage preparations was developed.

EXPERIMENTAL

A SP 8000 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.), equipped with a SP 8300 fixed-wavelength detector (254 nm) and with a data system, was employed during the study. The detector range was set at 0.04 a.u.f.s. A reversed-phase column (RP-2, 10 μm, 25 cm × 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, U.S.A.) was used at 30°C with a mobile phase flow-rate of 1.5 ml/min. Injections of 10 μl were made of all solutions to be analyzed.

Mobile phase

Phosphate buffer solution (0.05 *M*) was prepared from KH_2PO_4 to give an unadjusted pH of about 4.5. To 800 ml of buffer solution was added 200 ml of acetonitrile and without adjusting the final volume, the solution was filtered through a Millipore system using Reeve Angel glass fiber filters 934 AH (Whatman, Clifton, NJ, U.S.A.).

0021-9673/82/0000–0000/$02.75

Solutions

For the internal standard stock solution, 30 mg of dimethylphthalate was dissolved in 1 l of acetonitrile–water (1:1) (approx. 300 μg/ml).

Sample solutions

Capsules. An accurately weighed sample of homogeneous capsule contents, equivalent to 100 mg cloxacillin sodium, was dissolved in 100 ml water and stirred for 15 min. A 3-ml portion of the solution was transferred to a 10-ml volumetric flask, a 6-ml aliquot of internal standard stock solution was added and the volume made to 10.0 ml with distilled water.

The cloxacillin sodium standard (B.P. Cloxacillin Reference) solution and bulk drug solutions were prepared in a similar manner.

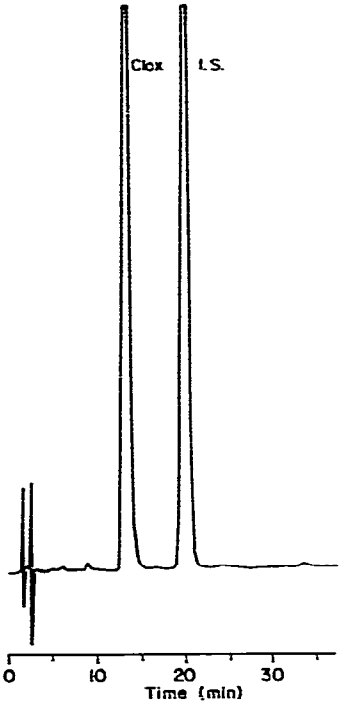
Oral Solution. The product was prepared according to manufacturer's instructions. The density of the solution was determined by accurately weighing a 5.0-ml aliquot. A portion of the solution equivalent to about 25 mg of cloxacillin sodium was weighed into a 25.0-ml volumetric flask and diluted with water to volume. A 3-ml portion of this solution was transferred to a 10-ml volumetric flask, 6 ml of internal standard stock solution were added and the volume adjusted to 10.0 ml with distilled water.

RESULTS AND DISCUSSION

A typical chromatogram of a cloxacillin capsule formulation is shown in Fig. 1. In addition a chromatogram of a mixture of oxacillin, cloxacillin, dimethylphthalate and dicloxacillin is shown in Fig. 2. The three isoxazole penicillins were well separated from each other and from the internal standard. Similarly, in the case of the oral solution which contained the preservatives, methyl and ethyl parabens, excellent separation of the formulation constituents was obtained. In the cloxacillin formulation examined, no interference from excipients or impurities was encountered. Table I shows the relative retention times (RRT) of cloxacillin, its common degradation products and other related compounds.

The degradation of cloxacillin to the penicilloic and penilloic acid derivatives was performed by standard procedures. The treatment of cloxacillin with alkali under controlled conditions yielded the penicilloic acid and the treatment of the penicilloic derivative under acidic conditions resulted in the formation of penilloic acid. The derivatives were not isolated.

The degradation of cloxacillin in acidic media (0.1 *M* hydrochloric acid) resulted in the formation of a product which was detected by the HPLC and exhibited a RRT of 4.01. Monitoring a parallel reaction by ultra-violet spectrometry established that the product had an absorption maximum of 344 nm, which is similar to that reported by Szawlowska and Borowicz[6]. A significant amount of the product was observed only after 1 h but its rate of decomposition was rapid and it had almost all disappeared within 2 h. The linearity of the detector response was established by injection of six solutions containing concentrations of cloxacillin ranging from 0.11–0.61 mg/ml with a constant concentration (0.18 mg/ml) of internal standard. When the ratios of the area counts of the cloxacillin divided by the area counts of the internal standard were plotted *vs.* concentration of cloxacillin, a straight line with a



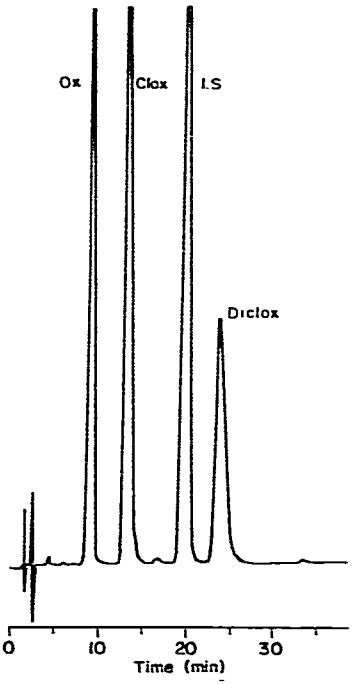


Fig. 1. HPLC chromatogram of cloxacillin (Clox) capsule formulation. I.S. = Internal standard (dimethylphthalate).

Fig. 2. HPLC chromatogram of oxacillin (Ox), cloxacillin (Clox), dicloxacillin (Diclox) and internal standard (I.S.).

coefficient of correlation of 0.9997 was obtained. The slope was 0.2006 $(mg/ml)^{-1}$ and the y intercept was -0.0265. The reproducibility of the analytical system was verified by analysing five different weighings of the same cloxacillin sample. The relative standard deviation of the area ratio of the cloxacillin peak to that of the internal standard peak was 1.73%.

TABLE I

RELATIVE RETENTION TIMES OF CLOXACILLIN, ITS DEGRADATION PRODUCTS AND OTHER RELATED COMPOUNDS

Compound	*Relative retention time**
Cloxacillin	1.00
Cloxacilloic acid	0.38
Cloxalloic acid	0.50
Dimethylphthalate (internal standard)	1.59
Methylparaben	0.86
Ethylparaben	1.50
Oxacillin	0.68
Dicloxacillin	1.82

* See text for chromatographic conditions.

TABLE II

HPLC AND CHEMICAL ASSAY OF CLOXACILLIN ORAL DOSAGE FORMULATIONS

Sample	*% of the label claim**	
	HPLC	*B.P. colorimetric method*
Bulk drug substance		
B.P. standard	90.5	90.5
House standard	90.7	90.6
Capsules		
Manufacturer 1 A	101.0	100.1
B	103.4	101.5
Manufacturer 2 A	99.2	94.8
B	98.1	95.0
Manufacturer 3 A	97.3	98.3
B	96.6	96.5
Manufacturer 4 A	97.7	99.3
B	89.0	88.7
Manufacturer 5 A	103.3	102.1
B	99.9	100.7
Manufacturer 6	95.3	96.5
Oral solution		
Manufacturer 2	102.0	102.2

* Average of triplicate determinations. All results relative to B.P. standard.

The HPLC method was applied to the analysis of 11 capsule formulations and one oral solution formulation of cloxacillin sodium. For comparison, the same samples were also analyzed by the B.P. colorimetric method. The results are summarized in Table II. Excellent correlation between the two methods was obtained.

All formulations examined revealed the presence of trace amounts (approx. 0.1% as cloxacillin) of two extraneous compounds (see Fig. 1). A comparison of the RRTs of these unknown compounds with the RRTs of the penicilloic and penilloic acid derivatives of cloxacillin implied the unknowns and these two degradation products were the same. As the amounts detected were so small as to be insignificant, further confirmatory identification was not undertaken. Neither the house standard nor the B.P. standard contained detectable amounts of these extraneous moieties.

The described HPLC method is rapid, precise and accurate for the analysis of cloxacillin bulk drug substance and solid and liquid oral dosage forms. Moreover, with no modifications, the method should be applicable to the analysis oxacillin and dicloxacillin formulations.

REFERENCES

1 *British Pharmacopoeia 1980*, Her Majesty's Stationery Office, London, 1980.
2 H. Bundgaard, *J. Pharm. Pharmacol.*, 26 (1974) 385.
3 *Code of Federal Regulations, Title 21, Part 440*, U.S. Government Printing Office, Washington, DC, 1981.
4 P. Celleti, G. P. Moretti and B. Petrangeli, *Farmaco, Ed. Prat.*, 27 (1972) 688.
5 E. S. A. Ibrahim, Y. A. Beltagy and M. M. A. El-Khalek, *Talanta*, 24 (1977) 328.
6 H. Szawlowska and P. Borowicz, *Chem. Anal. (Warsaw)*, 23 (1978) 821.