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Note

High-performance liquid chromatographic determination of dicloxacillin in presence of its degradation products

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Recently the need for a rapid, reliable method for determination of cloxacillin formulations led to the development of a high-performance liquid chromatographic (HPLC) assay procedure[1], which gave results that were in excellent agreement with those obtained by the *British Pharmacopoeia* (BP) colorimetric method[2].

As predicted by the authors[1], the HPLC method is directly and satisfactorily applicable to the analysis of oxacillin in the presence of its degradation products, but, contrary to their expectations, the method required adjustment for detection of the major product of acid degradation of dicloxacillin*, which was not detected under the original conditions. The procedure herein reported thus allows determination of dicloxacillin in the presence of its products of degradation in both basic and acid media.

EXPERIMENTAL

An SP 8000 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.), in conjunction with an SP 8440 variable-wavelength detector and 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 for all HPLC determinations.

The temperature was maintained at 30°C, the detector range set at 0.04 a.u.f.s. and the mobile phase flow-rate was 1.5 ml/min.

The injection sample size was 10 μl in all cases.

Mobile phase

To 750 ml of buffer solution (0.05 *M* potassium dihydrogen phosphate, pH 4.5) were added 250 ml of acetonitrile and the resulting solution was filtered through a Millipore system using Reeve Angel glass fiber filters 934 4H (Whatman, Clifton, NJ, U.S.A.).

Internal standard stock solution

An accurately weighed sample of *ca.* 30 mg of dimethylphthalate was dissolved in 1 l of acetonitrile–water (1:1).

* In the earlier publication[1] the nature of the cloxacillin product was not specifically mentioned, only an absorption maximum of 344 nm having been reported.

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Standard solution

In a 50-ml volumetric flask, *ca.* 10 mg of U.S.P. Reference Standard were accurately weighed and 15 ml of internal standard stock solution were added. The resultant was made up to volume with distilled water. The final solution was sonicated for 10 min.

Formulation solution

In a 50-ml volumetric flask, an amount of homogeneous capsule contents equivalent to 10 mg of dicloxacillin sodium was accurately weighed and 15 ml of internal standard stock solution were added. The resultant was made up to volume with distilled water. The final solution was sonicated for 10 min.

Dicloxacilloic acid

Dicloxacilloic acid was generated by the procedure[3,4] developed for preparation of benzylpenicilloic acid from Pen G, by treatment with aqueous sodium hydroxide.

Dicloxacillenic acid

Dicloxacillin sodium monohydrate (100 mg) was dissolved in 10 ml distilled water and an equivalent of hydrochloric acid (1 ml of a 7.8 mg/ml aqueous solution) was added slowly dropwise, with stirring, at room temperature. The resulting precipitate was filtered off under suction and dissolved in 10 ml of acetonitrile; half of an equivalent of hydrochloric acid was added and the resultant pH of the medium was 1.0. After stirring overnight, the acetonitrile solution was evaporated to dryness and the residue was dissolved in 10 ml of chloroform. The chloroform solution was washed with two 3-ml portions of water, dried over anhydrous sodium sulphate and filtered; the solvent was removed under reduced pressure. The white solid so obtained (97 mg, 81%) was 96.5% penicillenic acid by HPLC analysis at 337 nm. The product gave a strong positive reaction with Ellman's reagent, forming a bright yellow solution with a UV maximum at 410 nm, indicative of the presence of the sulfhydryl group.

RESULTS AND DISCUSSION

The HPLC system employed affords resolution of dicloxacillin from all of its products of degradation under both acidic and basic conditions; the degradation

TABLE I

RETENTION TIMES OF DICLOXACILLIN AND DEGRADATION PRODUCTS

	Representative retention time (min)	*Relative retention time*
Dicloxacillin	7.6	1.00
Dicloxacilloic acid	4.2	0.55
Dicloxilloic acid	3.1	0.41
Dicloxacillenic acid	17.5	2.30
Dimethylphthalate (IS)	5.2	0.68

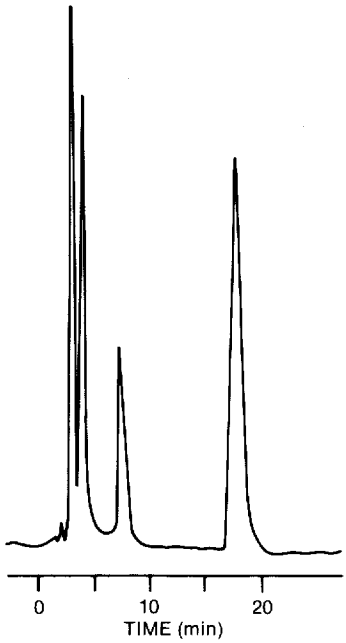

Fig. 1. HPLC chromatogram of dicloxacillin and its degradation products. Peaks: 1 = dicloxilloic acid; 2 = dicloxacilloic acid; 3 = dicloxacillin; 4 = dicloxacillenic acid.

products are also well separated from the internal standard used (see Table I and Fig. 1).

The linearity of the detector response was established by the injection of four solutions —two injections of each solution— ranging in dicloxacillin sodium concentration from 0.097 mg/ml to 0.427 mg/ml, internal standard concentration being maintained at 0.009 mg/ml. A straight line plot was obtained (coefficient of correlation, 0.9997; slopes, 0.0488; intercept, −0.0067) when the ratios of the area counts for dicloxacillin, divided by those for the internal standard, were plotted against the concentration of dicloxacillin sodium.

The coefficient of variation of the ratio of area count for dicloxacillin relative to that of the internal standard, for five different weighings of the same dicloxacillin sample, was 1.0%.

The assay method haas been successfully applied to the analysis of six different capsule formulations on the Canadian market, whose potencies were found to accord with label claim.

The only impurity detected in all formulations, as well as in the USP standard (1–2%), had a retention time of 3.0 min, which corresponds closely with that of the prime degradation product of the penicilloic acid, namely, the penilloic acid, dicloxilloic acid, formed by decarboxylation. When the product of basic hydrolysis was left in the reaction medium (see Experimental) overnight at room temperature, and examined by HPLC, the sole additional product observed —accompanying residual dicloxacillin and its product of β-lactam ring hydrolysis (dicloxacilloic acid)— appeared as a peak of retention time 3.1 min, in proportion comparable with that of

the initial hydrolysis product; no peak in the vicinity of 4 min, corresponding to dicloxacilloic acid, was ever observed either in formulations or dicloxacillin standard.

The clean efficient formation of dicloxacillenic acid under acid conditions was surprising in view of the widely recognized instability of the corresponding compounds derived from other penicillins[5] and the attendent difficulty of observing penicillenic acids unless they are stabilized by complexation with heavy metal ions[6]; penicillenic acids have been reported to degrade fairly rapidly to penillic and penicilloic acids[5]. Indeed, the published analytical profile on cloxacillin sodium[7] suggests reaction of the free thiol group of the penicillenic acid with Ellman's reagent in order to facilitate determination. However, in our current studies, the penicillenic acids of all three isoxazole penicillins have been isolated in excellent yield and high purity from acid treatment of the parent penicillins (oxacillin, cloxacillin and dicloxacillin) and have been assessed by HPLC with UV detection at 337 nm.

Since the yield and purity of the three products increase with the level of chlorine substitution in the parent isoxazole, dicloxacillenic acid has been selected for further study of its detailed degradative pathways. This subject is of special interest in view of the implication of sulfhydryl compounds in allergic response to penicillin therapy, and the results of this aspect of our continuing research will be reported elsewhere.

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