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Letter to the Editor

Assay for cloxacillin in human serum utilising high-performance liquid chromatography with ultraviolet detection

Sir,

Cloxacillin is a semi-synthetic β-lactamase-resistant penicillin. It is widely used in long-term prophylactic treatment against a wide range of infections and its assay is frequently required. However, at present only a few assay methods for cloxacillin using high-performance liquid chromatography (HPLC) are available [1,2]. We thought that its assay could be simplified and improved by the use of an alternative chromatographic column and mobile phase, the use of rapid solvent evaporation and the use of an internal standard. This prompted us to establish this assay method for cloxacillin in biological samples.

EXPERIMENTAL

Materials

Acetonitrile (HPLC grade) and dichloromethane (HPLC grade) were obtained from Water Assoc. (Milford, MA, U.S.A.). Cloxacillin and nafcillin were the United States Pharmacopeia (U.S.P.) reference standard (Rockville, MD, U.S.A.). All other chemicals were of analytical grade. Standard solutions (500 μg/ml) of cloxacillin sodium and nafcillin sodium were prepared in deionized water and could be stored for one month at $-20°$C.

Procedure

Serum (500 μl) was taken into a small glass centrifuge tube, and 15 μl of internal standard (equivalent to 7.5 μg of nafcillin) were added. The solution was acidified with 50 μl of 1 *M* sulphuric acid and mixed. After 30 s, 2 ml of dichloromethane were added and the contents of the tube were mixed for 2 min prior to centrifugation at 1130 *g* for 2 min at 25°C. The organic layer (1.8 ml) was transferred to a second tube and evaporated to dryness under nitrogen.

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The residue was dissolved in 200 μl of the mobile phase [acetonitrile–10 m*M* ammonium acetate in deionized water (20:80, v/v)], and 10–20 μl were injected (Waters HPLC system with a Model 6000A pump, a Model U6K universal injector, a Model Lambda Max 481 variable-wavelength UV detector, a Waters stainless-steel column, 30 cm × 4 mm I.D., of μBondapak C_{18}, particle size 10 μm). No guard column was used. The flow-rate was 2 ml/min. The detector was set at 254 nm and 0.01 a.u.f.s.

The standard curve of cloxacillin and internal standard showed linearity from 0.01 to 160 μg/ml of solution. Linear regression analysis ($y = mx \pm c$) with a coefficient of correlation (r) of 0.9990 indicates excellent agreement. The method was found to be precise and selective. The inter-assay precision of the method was assessed ($n = 10$) by repeated analysis of serum specimens containing 5 μg/ml cloxacillin. We found the coefficient of variation of cloxacillin to be 7.5% for 5 μg/ml and 6.5% for 10 μg/ml, respectively.

We mixed known amounts of cloxacillin and nafcillin with drug-free serum. The compounds were isolated from the serum mixtures by the procedure described. Fig. 1 shows the elution pattern of cloxacillin and nafcillin in human serum.

We measured the cloxacillin levels in the serum of nine healthy human sub-

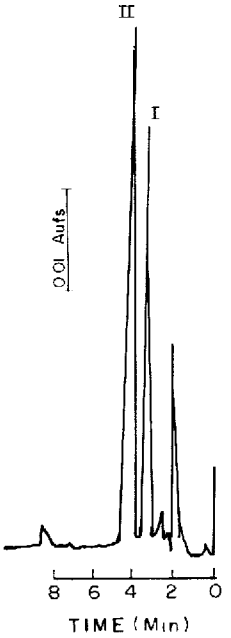

Fig. 1. Chromatogram of cloxacillin and nafcillin in human serum. The injection volume (20 μl) contained 0.9 μg of cloxacillin and 1.8 μg of nafcillin. Peaks: I = cloxacillin (3.2 min); II = nafcillin (4.1 min).

TABLE I

HUMAN SERUM LEVELS OF CLOXACILLIN

Nine volunteers were each given a single oral dose of 500 mg of cloxacillin.

Time of blood collection (h)	Concentration (mean ± S.D.) (μg/ml)
0.00	0.00 ± 0.00
0.25	4.00 ± 1.00
0.50	10.00 ± 6.63
0.75	12.24 ± 6.30
1.00	12.07 ± 5.44
1.50	9.16 ± 4.23
2.00	8.47 ± 3.27
3.00	5.49 ± 3.62
4.00	2.68 ± 1.56
5.00	1.35 ± 0.72
7 00	0.66 ± 0.46
8.00	0.52 ± 0.28
12.00	0.28 ± 0.18
24.00	0.15 ± 0.14

jects given a single dose of 500 mg of cloxacillin orally. Blood samples were collected via intraveneous canulla at various intervals (Table I). The peak serum level of cloxacillin was found to be 12.24 ± 6.30 μg/ml at 45 min after administration of the drug.

The present method has several advantages over the previously reported HPLC methods for analysis of penicillins [1,2]. It is accurate, as nafcillin was used as an internal standard. We used the technique of nitrogen evaporation for quick preparation of many samples. We obtained the best results by using a μBondapak C_{18} column and acetonitrile–aqueous ammonium acetate (20:80, v/v) as the mobile phase.

Cloxacillin is 94–96% bound to serum protein [3], so the drug was freed from serum protein by sulphuric acid and then extracted with dichloromethane. The recovery was ca. 95% for both cloxacillin and nafcillin. The efficiency of the column was nearly 100%, as assessed by using known amounts of cloxacillin and nafcillin. The selectivity of this method was such that no endogenous materials from the biological samples interfered with the assay of cloxacillin. This

technique should be useful for the measurement of cloxacillin levels in biological samples, such as serum, urine, plasma and drugs, after appropriate modifications.

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