Detection of Ampicillin Contamination in Nitrofurantoin Preparations by High Pressure Liquid Chromatography

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Abstract □ Ampicillin as a contaminant in nitrofurantoin capsules and tablets was determined by high pressure liquid chromatography. The method uses a strong anion-exchange column, 0.01 *M* KH_2PO_4 buffer, and an ampicillin working standard as reference. Identity of ampicillin was verified by assaying spiked samples of nitrofurantoin. The method is quantitative over the range 1–10 mcg. of ampicillin.

Keyphrases □ Nitrofurantoin tablets and capsules—analysis of ampicillin contamination, high pressure liquid chromatography □ Ampicillin contamination in nitrofurantoin tablets and capsules—analysis, high pressure liquid chromatography □ High pressure liquid chromatography—analysis, ampicillin contamination in nitrofurantoin capsules and tablets

Ampicillin, 6-[D(−)-α-aminophenylacetamido]penicillanic acid, is a semisynthetic penicillin, first prepared by Doyle *et al.* (1). The anhydrous and trihydrate forms and the sodium salt are widely used therapeutically. It has been shown to be inhibitory at low concentrations against a wide variety of both Gram-negative and Gram-positive bacteria (2–4). Because it is manufactured and formulated into dosage forms by many drug companies that also produce other drugs, cross-contamination is a possibility. This situation causes concern, particularly since small quantities of penicillin derivatives may cause allergic reactions (5–7). As part of its antibiotic certification program, the Food and Drug Administration (FDA) has developed and used tests (8) to check cross-contamination of penicillin in other pharmaceuticals, but these tests do not detect ampicillin.

A test to determine ampicillin contamination of nitrofurantoin became necessary when it was suspected as a contaminant in samples submitted to the National Center for Antibiotic Analysis for analysis. Attempts to recover ampicillin added to nitrofurantoin by the standard procedures (8) were unsuccessful because ampicillin, being amphoteric, could not be extracted into amyl acetate from an acid solution. A test based on liquid chromatography was developed.

A strong anion-exchange column was selected because ampicillin has an anion-exchangeable carboxylic group and is water soluble. Of various buffers and solvent systems tried, 0.01 *M* KH_2PO_4 (pH 6.5) buffer was optimal. This mobile phase separated nitrofurantoin and ampicillin and simultaneously was used to identify and quantitate ampicillin. The reliability of the method was verified by assaying spiked samples of nitrofurantoin capsules and tablets.

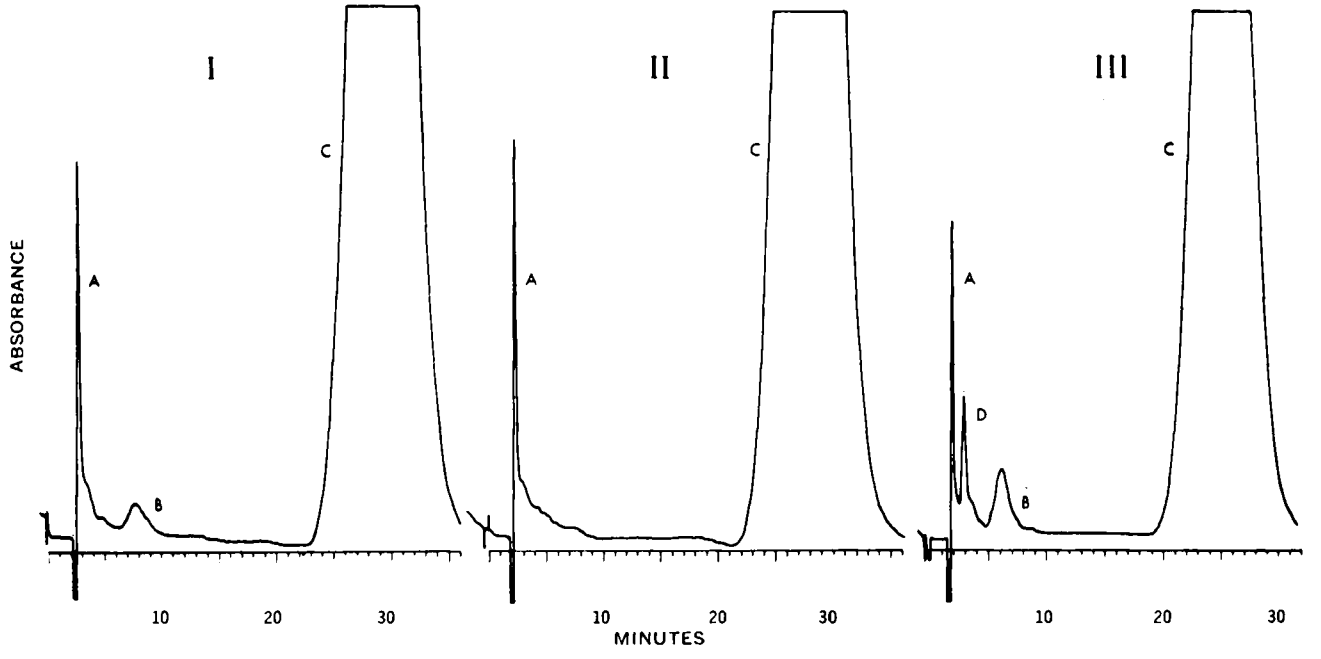

Figure 1—*Chromatogram of (I) nitrofurantoin capsules spiked with 0.1 mg. of ampicillin per dose, (II) uncontaminated nitrofurantoin capsules, and (III) commercial nitrofurantoin capsules contaminated with ampicillin. Key: A, solvent; B, ampicillin; C, nitrofurantoin; and D, unidentified impurity.*

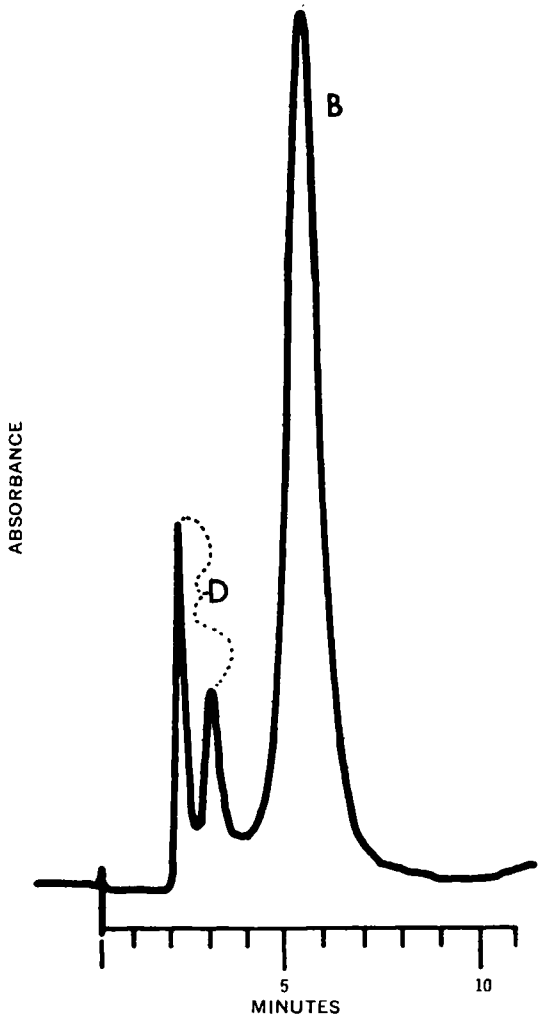

Figure 2—*Chromatogram of a contaminated bulk sample of ampicillin trihydrate. Key: B, ampicillin; and D, unidentified impurities.*

EXPERIMENTAL

Materials—A liquid chromatograph[1] was used with a UV detector; the flow rate was approximately 1.0 ml./min. A strong anion-exchange column[2], 1.0 m. by 2.1 mm. i.d., was obtained already prepared. Reagents were 0.01 *M* KH_2PO_4 buffer, pH 6.5; chloroform, reagent grade; and ampicillin trihydrate, FDA working standard.

Assay Procedure—Place the contents of 10 nitrofurantoin (50–100 mg.) capsules, or the powder from 10 ground tablets, into a 15-ml. centrifuge tube. Add 5.0 ml. of water and shake. Centrifuge the suspension, and decant the supernatant layer through a funnel containing a wad of glass wool into a second 15-ml. centrifuge tube. Add 10.0 ml. of chloroform to the second tube and shake vigorously. Allow the aqueous and chloroform layers to separate; then inject 5.0 μl. of the aqueous layer into the chromatograph for the ampicillin analysis.

Using an ampicillin reference standard at a concentration of 2.0 mg./ml. in distilled water, prepare a standard curve over a 1.0–10.0-mcg. range by chromatographing aliquots ranging from 0.5 to 5.0 μl. Obtain the peak area of each chromatogram by the method of peak height times width at half-height, and calculate the results by the following formula: micrograms ampicillin per dose unit tested = [(micrograms ampicillin from standard curve) (microliters total volume of solution)]/[(number of dose units tested) (microliters sample solution injected)].

RESULTS AND DISCUSSION

This method is quantitative for gross contamination of ampicillin in nitrofurantoin over a range of 1.0–10.0 mcg. of ampicillin. The standard curve is linear over this range. The constant retention time for ampicillin is an index of its identity.

The aqueous ampicillin solution must be extracted with chloroform to remove undefined chromophoric substances which gave a retention time very close to that of ampicillin. Because ampicillin is very water soluble, recovery was satisfactory. As shown in Fig. 1 (I), the ampicillin peak is well resolved from that of nitrofurantoin.

The sensitivity of this procedure is limited by the fact that 1.0 mcg. is the smallest amount of ampicillin that can be detected and quantitated accurately in the instrument used. Control samples were spiked at this level with 5.0 ml. of an aqueous solution containing 0.2 mg./ml. of ampicillin. A contamination of 0.1 mg./nitrofurantoin dose was assumed, so it was necessary to use 10 doses/5.0 ml. of water. The chromatogram of a sample of nitrofurantoin spiked at 0.1 mg. of ampicillin/dose is shown in Fig. 1 (I). Recovery of ampicillin from the spiked control sample was 94.0%.

Figure 1 (II) shows a chromatogram of nitrofurantoin capsules in which no ampicillin was detected. Figure 1 (III) shows a chromatogram of a sample of a nitrofurantoin capsule that was found to be contaminated with 158 mcg. of ampicillin/capsule dose.

Despite the limited sensitivity of this procedure, it provides useful data that cannot be obtained by other known methods. This procedure also makes it possible to perform identity studies by collecting fractions corresponding to the respective peaks in the chromatogram. The peaks preceding that of ampicillin could be collected separately or together and subjected to further analysis. The procedure may also be used to determine ampicillin in other drug formulations if excipients are removed in the preparative phase of the method to avoid exceeding the load capacity of the strong anion-exchange column as stated by the manufacturer.

This method has been used to examine ampicillin samples for purity. The chromatogram for the ampicillin FDA working standard and some commercial bulk samples showed a single, symmetrical peak, whereas other commercial ampicillin bulk samples yielded multiple-peaked chromatograms (*e.g.*, Fig. 2), indicative of the presence of impurities. These findings will be further investigated.

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[1] Model 830, E. I. duPont de Nemours and Co., Wilmington, Del.
[2] E. I. duPont de Nemours and Co., Wilmington, Del.