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Note

Separation and semi-quantitative determination of ampicillin polymers in ampicillin bulk preparations by means of thin-layer chromatography

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Polymerization products of ampicillin have been reported to possess strong antigenic properties, as shown in animal experiments[1–4], and may therefore play a part in the elicitation of some clinical allergic reactions to ampicillin. A recent clinical study by Parker and Richmond[5] indicated that the use of so-called "polymer-free" ampicillin may reduce the incidence of exanthematic adverse reactions to ampicillin preparations.

Because of the immunological effects of ampicillin polymers, their presence in clinically used ampicillin preparations should be controlled and kept at the lowest possible level. Larsen and Bundgaard[6] developed a sensitive high-performance liquid chromatographic (HPLC) procedure, which separates and quantifies the individual ampicillin polymers, ranging in size from the dimer to the octamer. Using this method substantial amounts of polymeric substances were found to be present in some clinically used ampicillin sodium bulk samples.

From this perspective we found a need for a simple and convenient thin-layer chromatographic (TLC) method to identify and quantify di- and polymeric impurities in ampicillin bulk preparations. This paper describes a TLC procedure which allows a simple and rapid separation and identification of ampicillin polymers in the presence of ampicillin and the penicilloic acid of ampicillin. Furthermore, the method allows a semi-quantitative assessment of polymeric contamination in clinically used ampicillin preparations.

EXPERIMENTAL

Materials

Ampicillin sodium (Doktacillin®) was purchased from Astra, Södertälje, Sweden. Before use ampicillin sodium was precipitated twice at isoelectric pH to remove di- and polymeric impurities[7]. The di-, tetra-, hexa- and octamers of ampicillin were isolated from a 20% (w/v) aqueous solution of ampicillin sodium, pH 8.5, kept at room temperature for 3 days as described previously[8]. The purity of the polymers was found to be at least 90% as determined by reversed-phase HPLC[6]. α-Aminobenzylpenicilloic acid was prepared by alkaline hydrolysis of ampicillin sodium. All other chemicals used were of analytical grade.

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Preparation of standard solutions

Standard solutions of ampicillin sodium, penicilloic acid of ampicillin, and di-, tetra-, hexa- and octamers of ampicillin were prepared in 0.05 *M* citrate buffer, pH 6.5, to contain from 10 μg/ml to 30 mg/ml.

Thin-layer chromatography

Commercially available precoated 20 × 20 cm silica plates (Kieselgel 60; Merck, Darmstadt, G.F.R.) were used. An aliquot of 1 μl of each of the solutions was applied to the plate. The mobile phase consisted of *n*-butanol–formic acid–water (80:3:1). In a saturated chamber the plates were developed to a height of 15 cm in about 165 min. The plates were dried at 100°C for 15 min and visualized after spraying with 1% starch solution–acetic acid–0.1 *N* iodine solution (100:8:1). The presence of the different compounds is demonstrated by the appearance of pale spots on a blue background.

RESULTS AND DISCUSSION

Table I shows the R_F values of ampicillin, the penicilloic acid of ampicillin and the polymerization products investigated. Several experimental conditions other than those indicated in the Experimental section were tried, *e.g.*, variation in the composition of the mobile phase and the visualization reagent. With the selected system optimal separation of the different compounds was obtained and a minor tailing was observed only at the spots corresponding to solutions with concentrations greater than 1%.

TABLE I

R_F VALUES OF AMPICILLIN AND SOME OF ITS DEGRADATION PRODUCTS

The R_F values are average values from five plates.

Compound	R_F
α-Aminobenzylpenicilloic acid	0.10
Ampicillin	0.28
Dimer	0.34
Tetramer	0.42
Hexamer	0.47
Octamer	0.55

After spraying with the starch–acetic acid–iodine reagent, the blue background of the TLC chromatograms fades as a function of time, and it was found that the chromatograms should be inspected within 10 min after visualization. The sensitivity of the method was determined by spotting dilutions of the standard solutions in amounts down to 0.01 μg. The limit of detection was found to be 0.05 μg for each compound.

By chromatographing the compounds in admixture it was confirmed that the chromatographic system allows separation of the individual compounds. Furthermore it was found that the size and colour intensities of the spots corresponding to

equal amounts of ampicillin and the polymerization products were almost identical thus allowing for semi-quantitative comparison between the compounds.

The method was applied to different pharmaceutical formulations of ampicillin clinically used in Denmark. By comparing the size and intensity of the spots from the sample solution with those obtained from standard solutions of the isolated ampicillin polymers, it was possible to detect impurities to concentrations as low as 0.5% (w/w) in these formulations. The results obtained in this way were in the same order of magnitude as those described by Bundgaard[7] using an HPLC method.

Even without standard solutions of polymers it is possible to access the approximate amounts of di- and polymers present in an ampicillin samples. When spotting different dilutions of the aqueous ampicillin sample solution, the amounts of di- and polymeric substances in the sample can be calculated by use of the limit of detection.

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