base catalysis of the primary amine produces the thioester which may be converted to the stable penicilloylamide.

Although several pathways for the formation of penicilloylamide antigenic determinant have been proposed (see reviews by Schwartz, 1969; Schneider, 1970), the present results indicate the possibility that *in vivo* penicilloylation of a hydroxyl and/or thiol group suitably placed on protein should occur rapidly with the assistance of general acid-base catalysis toward penicillin β-lactam to yield the corresponding penicilloyl esters, which are then rapidly converted to penicilloylamide by nucleophilic attack by a proximate ϵ-amino-group of lysine on protein. Schneider & de Weck (1968) have excluded such a possibility for the thiol reaction *in vivo* on the grounds that no protein with an *N*-terminal cysteine has been identified. However, protein with an *N*-terminal serine or cysteine is not necessarily required, what is needed is the combination of three-dimensionally and closely located functional groups on protein.

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A simple, new method for testing impurities in cephalexin

The British Pharmacopoeia (1973) specifies a paper electrophoresis method to measure the phenylglycin and 7-AMCA (7-amino-3-methyl-3-cephem-4-carboxylic acid) impurities present in cephalexin (7-(D-α-amino-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid). However, not all drug quality control laboratories possess electrophoresis equipment. Therefore we have developed a comparable but simpler method.

The method is based on that of Dévényi (1970). 20 × 20 cm chromatography plates (coated with 50% Dowex 50 × 8, Fixion, Chinoin, Budapest) were used in Na^+ cycle (Dévényi & Zoltán 1970). The plates were washed for 48 h according to the method of Decker (1951) with sodium citrate, pH 3·28, 0·02 N Na^+ (see Fig. 1), and then dried at room temperature (when dry the plates can be stored at room temperature for several months).

The buffer solutions used were:

	pH 5·28 (0·35 N Na^+)	pH 4·59 (0·35 N Na^+)	pH 3·42 (0·2 N Na^+)
Citric acid monohydrate	24·6 g	19·35 g	14·1 g
Sodium hydroxide	14·0 g	11·0 g	8·0 g
Hydrochloric acid 37% (sp. gr. 1·19)	6·5 ml	9·4 ml	12·3 ml
Each solution was made up to 1000 ml with ion-free distilled water			

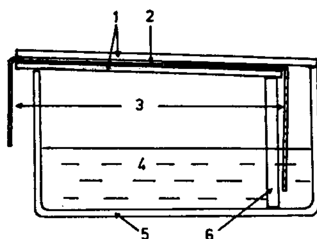

FIG. 1. Washing process of chromatography plates: 1 glass plates, 2 Fixion chromatography plates, 3 filter paper strips, 4 washing buffer, 5 glass dish, 6 glass tube.

The ninhydrin spray reagent used was prepared as follows. Solution A: ninhydrin (0·4 g) is dissolved in ethanol (100 ml) and then acetic acid (20 ml) and pyridine (4 ml) are added. Solution B: cupric nitrate trihydrate (1·0 g) is dissolved in ethanol (100 ml). Just before use 100 ml of solution A is mixed with 6 ml of solution B.

0·08 ml of the 1% aqueous solution of cephalexin and 0·01 ml of 0·02% aqueous solutions of phenylglycin and 7-AMCA were applied to the prepared plate. The plates were then developed in one of the three buffer solutions at room temperature for approximately 4–5 h so that the solvent front travelled a distance of ~18 cm. The plates were then dried in a stream of hot air and the components visualized by spraying with the ninhydrin reagent, and heating to 60° for 10 min.

The following R_F values represent the average of six measurements taken with mm accuracy:

pH (± 0·05) of the developing mixture:	3·42	4·59	5·28
Cephalexin	0·10 ± 0·03	0·27 ± 0·04	0·34 ± 0·05
7-AMCA	0·23 ± 0·04	0·46 ± 0·03	0·50 ± 0·04
Phenylglycin	0·30 ± 0·06	0·58 ± 0·03	0·67 ± 0·04

The two methods proved to be comparable for testing the impurities in cephalexin. The ion-exchange layer chromatography is suitable for the qualitative testing of amphoteric substances (eg, amino-acids, amphoteric antibiotics, alkaloids and other natural or synthetic organic derivatives) (Dévényi, 1970; Dévényi, Báti & Fábián, 1971; Dávid & Takácsy, 1974). It follows from the characteristics of cation-exchange resin that the method is also suitable for purity testing of non-amphoteric organic bases, if the dissociation constants of the basic impurities are sufficiently different.

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