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Note

A novel thin-layer chromatographic system and its application to the separation of some cephalosporin compounds

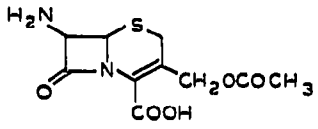
During work on the chromatographic separation and semi-quantitation of various intermediates encountered in the synthesis of cephalosporin antibiotics, a thin-layer chromatographic (TLC) system was required that would separate 7-aminocephalosporanic acid [7-ACA (I)] from related compounds.

Attempts to chromatograph (I) on pre-prepared silica gel glass-backed layers using systems containing water and organic solvents such as ethanol, methanol, butanol and ethyl acetate are generally not very successful. Many systems show no movement of the compound and, where movement is effected, quantitation is made difficult by slow running and/or streaking. Although the running properties of (I) markedly improve when large amounts of water are used with acetonitrile, such systems are unsatisfactory as two solvent fronts appear during development.

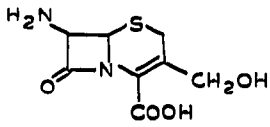
Other workers have shown that (I) can be successfully separated from related compounds using reversed-phase TLC[1], electrophoresis and paper chromatography[2], but as Merck glass-backed Silica Gel F_{254} layers have the advantages of general convenience, fluorescence and ease of handling, further work was undertaken with the aim of successfully using highly aqueous solvent systems with these plates.

Silica gel layers on glass and plastic are largely destroyed by development in pure water. However, we have found that the addition of a small amount of a simple inorganic salt such as sodium chloride to water, prior to development of a Merck pre-coated Silica Gel F_{254} plate, leaves the thin layer intact. This may be due to the reduced solubility of the silica and/or binder in sodium chloride solution compared to water, since plate development in a saturated aqueous solution of this shows the same effect. The addition of inorganic salts to TLC systems containing organic solvents has been reported to give improved separation of some pharmaceutical products compared to the untreated solvents[3]. However, such systems are found to be unsatisfactory for the separation of (I) from other compounds. These workers also reported that great differences in running properties occur when one salt is replaced by another in the same solvent system. We have replaced sodium chloride solution with caesium chloride and lithium chloride solutions of the same concentration and found that only minor changes in R_F values of 7-aminocephalosporins occur. When (I) is developed in the sodium chloride system, it shows considerable movement as a reasonably compact spot without on-plate decomposition and conditions were optimised for the separation of (I) from some related cephalosporins.

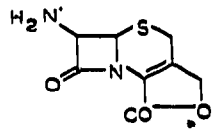
An example of the application of this TLC system is the separation of (I) from its products of acid hydrolysis. In dilute acid solution (I) breaks down to form deacetyl-7-aminocephalosporanic acid (II) which it is suggested[2] then forms the corresponding lactone (III).



I



II



III

Experimental

A 200-mg sample of (I) in 100 ml of 0.1 *N* HCl is stirred mechanically at 45° for 2 h. The solution is cooled to room temperature, 5 ml is diluted to 10 ml with phosphate buffer, pH 7 (2.65 g of potassium dihydrogen phosphate and 5.31 g of dipotassium hydrogen phosphate in 100 ml of water) and 10 μl of this solution plus reference loadings of (I), (II) and (III) in phosphate buffer are then spotted on to a Merck Silica Gel F_{254} plate. This is then developed in aqueous 0.5 *M* sodium chloride solution until the solvent is within 1 cm of the top, development time being 2 h.

After drying the plate, initial visualisation is effected by UV light (254 nm). R_F values for the observed spots are given in Table I.

TABLE I

R_F VALUES OF 7-ACA AND RELATED COMPOUNDS IN 0.5 *M* NaCl SOLUTION

Compound	R_F
Deacetyl-7-ACA	0.58
7-ACA	0.44
7-ACA lactone	0.29
Reaction solution components	0.58, 0.44, 0.29

Alternative visualisation of the plate is achieved by spraying the plate with a modified form of a reagent described for the detection of primary amines on TLC plates[4]. This consists of two solutions: 1% 2,5-dimethyloxytetrahydrofuran in glacial acetic acid and 1% dimethylaminobenzaldehyde in 15% conc. hydrochloric acid in glacial acetic acid, mixed just before use in the ratio 1:2. After heating the plate at 100° for 3 min, the compounds show up as red spots on a yellow background. The limit of detection of the named compounds using this reagent is 0.05 μg.

Conclusion

The novel TLC system described gives good separation of the acid hydrolysis products of (I) and it is suggested that the system may find application in other situations where the separation of polar compounds (such as (I)) using water on silica gel plates is required. The system combines useful properties of water such as great solvating power and high dielectric constant with the well characterised separating powers of silica gel. For other applications, the pre-coated plates can be washed in water–methanol (6:4) solution to remove water-soluble material which may otherwise interfere with the chromatographic separation.

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