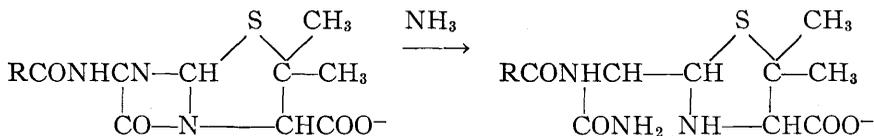


A TEST FOR PENICILLINS ON CHROMATOGRAMS AND IN SPOT TESTS^{1, 2}. A simple procedure for detecting small amounts of a penicillin on a paper chromatogram or in a spot test is described in this paper. A simple test has previously been described (Day, 1957) for penicillins, including a totally synthetic penicillin, α -formamidopenicillanic acid, of low antibiotic activity. Subsequently a similar test was described by Cox and Greenwell (1965) for phenethicillin. Except for the natural penicillins and some of the synthetic analogs shown to have high biological activity, which may be detected by bioassay, there have heretofore been no sensitive tests suitable for locating penicillins on paper chromatograms.

N-acylated thiazolidines, which include the penicillins, do not react with iodine in neutral solutions at an appreciable rate. However, thiazolidines which are not N-acylated do consume approximately 9 equivalents of iodine (Scudi and Woodruff, 1949). The test found applicable for the detection of penicillins on developed paper chromatograms begins by treating the paper to which the penicillin has been applied with ammonia. Ammonia cleaves the β -lactam ring of the penicillin, producing the α -amide of the corresponding penicilloic acid. Sodium salts of penicillin are cleaved quantitatively by ammonia in a few minutes (Mozingo and Folkers, 1949).



The developed paper chromatogram, to which 10–1000 micrograms of penicillin has been applied, is sprayed with aqueous ammonia (concentrated) and then suspended in a chamber over concentrated aqueous ammonia for approximately one hour. After removing the strip and allowing the ammonia to escape, it and a control strip, untreated with ammonia but otherwise developed in an identical manner, are passed through dilute iodine ($5\text{--}15 \times 10^{-4} \text{ N}$) in acetone (freshly prepared). As soon as the acetone has evaporated, the strips are sprayed with aqueous 1% starch – 1% potassium iodide. The penicillin appears as a white spot or zone on a violet background on the strip treated with ammonia, but not on the control. Easily oxidized impurities also appear in identical positions on both strips.

It is possible to detect 10 γ of either the penicillin or its penicilloic acid on Whatman No. 1 paper very readily. Amounts as small as 2 γ (3 Oxford Units of penicillin G) can be detected. Somewhat lower sensitivity is observed on heavier

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paper, such as Whatman 3MM. The order of addition of the starch-potassium iodide to the paper and iodine is not critical.—MARGARET GOLDMAN and RICHARD A. DAY, *Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221.*

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