

## The detection of amides on paper chromatograms

No convenient test appears to have been described for the detection of amides on paper chromatograms. In the case of an amide being ninhydrin-negative the only method of detection is the general test for N-H-containing compounds based on the formation of N-chloro-derivatives<sup>1-3</sup>. SOLOWAY AND LIPSCHITZ<sup>4</sup> described a colorimetric test for amides and nitriles, based on their reaction with hydroxylamine to give hydroxamic acids and amidoximes respectively, followed by the formation of coloured ferric complexes. It was found that this test could be modified for use as a spray reagent for the detection of amides on paper chromatograms. The method is as follows.

*Reagents.* Saturated solution of hydroxylamine hydrochloride in methanol and ferric chloride hexahydrate (1%) in ethanol.

*Procedure.* The paper was sprayed with hydroxylamine reagent with simultaneous drying of the reagent on the paper using a hot air drier. In order to apply a thick even coating of the reagent the paper was covered twice in this fashion. It was then hung in an oven at 100° for 20 min. Finally the paper was sprayed very lightly with ferric chloride reagent, when a positive test was the immediate production of a red colour stable over several hours.

SOLOWAY AND LIPSCHITZ<sup>4</sup> recommended the use of a solvent with a high boiling point, propylene glycol, since the reaction of amides with hydroxylamine proceeded better at elevated temperatures. In the adaptation to paper chromatograms it was found that the non-volatile solvent led to extensive diffusion of the spots with greatly lowered sensitivity. Furthermore the reaction proceeded well when the reactants were in the solid state. Hydroxylamine was therefore applied to the paper in a more volatile solvent which was evaporated as rapidly as possible after application.

As little as 50 µg of asparagine, glutamine, nicotinamide and isobutyramide could be detected after one-dimensional chromatography using this procedure; 30 mg amide was used in the colorimetric procedure<sup>4</sup>. In view of the wide applicability of the colorimetric test to amides, the procedure described here should also have wide applicability for non-volatile amides. For amides with melting points in the region of 100° or less, the reaction can be carried out at a lower temperature with heating for correspondingly longer periods.

The ureides allantoin and citrulline gave negative tests, while urea reacted anomalously giving a blue colour which faded rapidly. Several organic acids and amino acids gave weak positive reactions, so that the test can only be employed when standard markers are also chromatographed. The cyanoglucosides linamarin and lotaustralin (which contain a nitrile group) also reacted positively, but required longer heating (30 min) for optimum colour production.

*Plant Chemistry Division, D.S.I.R.,  
Palmerston North (New Zealand)*

J. G. ROBERTSON  
G. W. BUTLER

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