

A simple technique for detecting homogentisic acid on paper chromatograms

Paper chromatography has proved useful in the identification of homogentisic acid in the urine of alcaptonuric subjects¹. Such chromatograms are usually stained with Pauly's reagent or ammoniacal AgNO_3 . The use of the latter reagent has its own limitations. It gives rise to extra spots apart from homogentisic acid and its products, and the chromatogram turns brown after prolonged exposure. These defects have been largely eliminated by the use of a simple and specific colour reaction for homogentisic acid developed in this laboratory² and the results of this investigation are presented in this communication.

Collection and subsequent processing of the urine samples of alcaptonuric subjects are carried out as outlined earlier².

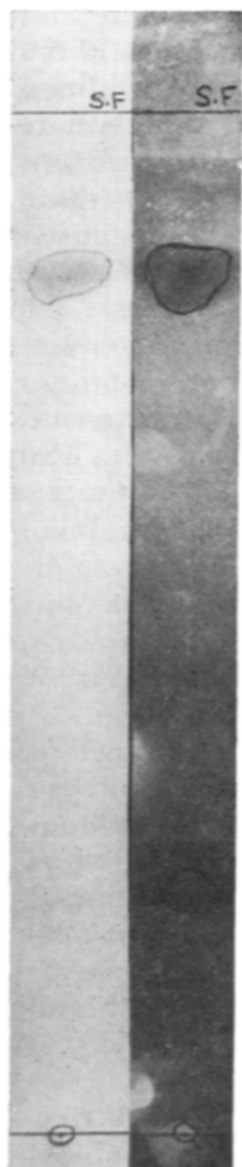


Fig. 1. Paper chromatogram of the urinary extract of an alcaptonuric subject. Left: developed with the new reagent; right: developed with ammoniacal AgNO_3 .

Paper chromatography of the urinary extract is carried out on Whatman No. 1 paper in butanol-acetic acid-water (4:1:5).

Chromatograms were stained with ammoniacal AgNO_3 and with the reagent developed by us. This reagent is prepared by mixing 5 ml of 0.01 % ethanolic CuSO_4 with 20 ml of ethanol and 10 ml of 0.01 N ethanolic NaOH.

The dried chromatograms were dipped into this reagent and allowed to remain there for 10 min for the development of a pinkish brown colour. The chromatograms were then air-dried.

It is clear from Fig. 1 that the new reagent developed by us for homogentisic acid has a distinct advantage over ammoniacal AgNO_3 . The chief objections to the use of ammoniacal AgNO_3 for developing the chromatograms, *viz.* the appearance of extra spots unrelated to homogentisic acid and its products and the turning of the chromatogram to brown colour after prolonged exposure, have been virtually eliminated by the use of this new developer. The chromatogram developed with the new reagent showed only one pinkish brown spot corresponding to homogentisic acid and which was clearly seen on a perfectly white background. However, the R_F value of homogentisic acid in our experiment was 0.85, which was slightly higher than reported earlier¹.

In earlier experiments, we tried the use of this reagent in aqueous medium. This resulted in the complete washing out of the spot corresponding to homogentisic acid. This has necessitated the use of the ethanolic solution of the reagent. The use of ethanol in this reagent is helpful in some manner in fixing the homogentisic acid in the paper during colour development.

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