

CHLORANILIC ACID AS A REAGENT IN THE
PAPER CHROMATOGRAPHY
OF THE SODIUM SALTS OF ORGANIC ACIDS

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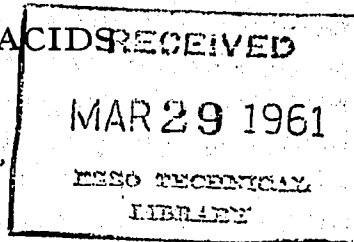
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INTRODUCTION

Chloranilic acid (2,5-dichloro-3,6-dihydroxy-*p*-quinone) was first applied in analytical chemistry by BARRETO^{1,2}, who used it for the determination of inorganic compounds such as calcium, barium, strontium and zinc. Later on the same author used it as a reagent in organic chemistry, obtaining good results with nitrogenous compounds such as caffeine, theobromine and theophylline³, coniine⁴ and nicotinamide⁵.

We have already applied chloranilic acid in the paper chromatography of inorganic compounds⁶, whereby a high sensitivity was obtained together with a very low specificity, so that it can be classified as an efficient chromatographic reagent. This efficiency was maximal for sodium, of which amounts as low as 0.2 μ g could be detected.

The two main problems in the paper chromatography of organic acids are the "tailing" of the spots and the loss of material due to volatilization (in the case of the two lower members of the fatty acids), which can both be avoided by using salts instead of the free acids. The ammonium derivatives are most frequently used^{7,8}, as they react readily with the pH indicators commonly employed as sprays^{8,9}. They are not so efficient, however, from the point of view of preventing volatilization. In this respect sodium salts are much more effective; they were used by BROWN¹⁰, BROWN AND HALL⁹ and HISCOX AND BERRIDGE¹¹.

In any case, the use of pH indicators for the localization of the isolated spots is not very satisfactory, as the colors are transient. By using the sodium salt in order to stabilize the spots, and localizing them by means of the reaction of chloranilic acid with the cation, a good sensitivity and a permanent record were obtainable.

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MATERIALS AND METHODS

Reagent. Chloranilic acid (0.1 % in ethyl ether) kept in a dark bottle.

Samples. Stock solutions of the sodium salts of the organic acids were prepared so that they contained 1 % of the cation. They were kept in the refrigerator and diluted as required.

Paper chromatography. Macherey-Nagel No. 261 filter paper was used, together with the solvent system of ISHERWOOD AND HANES⁷ (propanol-conc. ammonium hydroxide (70:30)). The chromatograms were run by the descending technique for 24 h (displacement of about 30 cm), or by the "dripping" technique for 48 h, in which case formate was taken as reference standard ($R_F = 1.0$).

Identification of the spots. The thoroughly dried chromatogram (120°/15 min) was put in an enamel tray and covered with a thin layer of the reagent. After a few seconds the reagent was returned to the bottle and the paper was washed twice with ethyl ether, dried (with hot air or in the oven at 120°) and observed under the U.V. lamp.

Sensitivity evaluation. As stated⁶, the sensitivity limit of chloranilic acid for the sodium ion is 0.2 μg . A sufficient volume of the samples was applied to the paper to yield that amount of the cation. It was then run as a descending chromatogram as described. In some cases the sensitivity was higher than the theoretical value, and smaller amounts were tested to ascertain the limit.

RESULTS AND DISCUSSION

The results obtained with the described technique are summarized in Table I. The R_F values found by us are not the same as those obtained by ISHERWOOD AND HANES⁷. This is partly due to differences in procedure (they used Whatman No. 1 filter paper), but mainly to the use of different cations (they used NH_4^+ and not Na^+).

TABLE I

SENSITIVITY OF CHLORANILIC ACID TOWARDS THE SODIUM SALTS OF SOME ORGANIC ACIDS IN DESCENDING PAPER CHROMATOGRAMS
The theoretical values are given between parentheses.

Sample	$\mu\text{g salt}$	$\mu\text{g acid}$	R_F	R_F^*
Carbonate	0.4	0.2	0.11	0.68
Formate	0.6	0.4	0.19	1.00
Acetate	0.7	0.5	0.14	0.87
Citrate	0.7	0.5	0.05	0.31
Oxalate	0.6	0.3	0.00	0.00
Tartrate	0.6	0.4	0.02	0.12
Pyruvate	1.0	0.7	0.14	0.77
Lactate	1.0	0.7	0.16	0.87
α -Ketoglutarate	0.8	0.6	0.13	0.68
Glycerophosphate	1.3	1.0	0.12	0.66
Nicotinate	1.0 (1.3)	0.8 (1.1)	0.13	0.90
<i>p</i> -Aminobenzoate	1.0 (1.3)	0.8 (1.1)	0.13	0.86

* With formate as reference substance in "dripping" chromatograms.

As has been stated, we preferred sodium because we had a very sensitive reaction for its identification, which not only yields a permanent record but is also simple and rapid. We also checked the ammonium salts (the sensitivity of chloranilic acid towards ammonium salts is about the same as for sodium salts), but some loss by volatilization was observed in the case of the formate and acetate spots.

Under the conditions we used, paper chromatograms of the sodium salts of organic acids show a single spot for each sample, with the exception of some impure batches of sodium acetate and sodium lactate, which presented a fake spot besides the true one. This led us to the assumption that no dissociation of the salt occurred during development.

Should dissociation occur, then in a medium containing such a high concentration of ammonium hydroxide as the solvent we used, at least three spots would have been found: one for the sodium salt itself, another for the newly formed ammonium derivative and yet another one for the resulting NaOH.

The R_F values for such possible contaminants differ considerably from those of the original samples, as can be seen in Table II.

TABLE II
COMPARISON OF THE R_F VALUES OF THE SODIUM SALTS OF SOME ORGANIC ACIDS
WITH THOSE OF POSSIBLE CONTAMINANTS

Salt	Na ⁺	NH ₄ ⁺
Acetate	0.14	0.78
Citrate	0.05	0.11
Oxalate	0.00	0.12
Tartrate	0.02	0.21
Hydroxide	0.13	—

As is to be expected, the sensitivity is proportional to the number of neutralized carboxyls and inversely proportional to the number of carbon atoms in the molecule. On the other hand, the sensitivity of chloranilic acid towards the sodium salts of nitrogen-containing organic acids, such as nicotinic and *p*-aminobenzoic acid, is higher than that expected from their sodium content. This is due to coupling of the reagent with the pyridine nucleus, in the first case, and with the aromatic amine, in the second.

The sensitivity of chloranilic acid towards carbonate was included in Table I, because of the occurrence of carbonate in the metabolic pathways of micro-organisms, and because this is the case in which the chromatographic separation of organic acids is most frequently used.

The high reactivity of chloranilic acid forbids the use of buffered papers and solvents, as well as of chelating agents such as EDTA (ethylenediaminetetraacetic acid, versene). The chromatograms must be thoroughly dried before development, in order to avoid ghost spots (due to traces of moisture). This is especially necessary when using ammonia-containing solvents, which are bound to cause heavily coloured

backgrounds. It must also be borne in mind that only neutral or basic solvent systems can be used, as acid solvents would cause decomposition of the sodium salts.

From what has been said it can be inferred that the main advantages of the use of the sodium salts for the paper chromatography of organic acids are the stabilization of the spots of the lower members (formic and acetic acids) as well as of carbonic acid, which frequently accompanies them, and the prevention of "tailing". Chloranilic acid is a very sensitive reagent for the localization of the spots; the procedure is rapid and easy, and a permanent record of the results is obtained.

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SUMMARY

The authors studied the use of chloranilic acid for the localization of sodium salts of organic acids separated by paper chromatography. Besides avoiding "tailing" of the spots and loss by volatilization of the lower members of the series, the use of sodium salts permits a very high sensitivity, as amounts as low as 0.2 μg of sodium are detectable. Sensitivity in relation to the acids was found to be proportional to the number of neutralized carboxyls and inversely proportional to the number of carbon atoms in the molecule. In the case of nitrogen-containing organic acids the sensitivity was higher than that expected, because chloranilic acid reacts with the N atom.

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