CHLORANILIC ACID AS A REAGENT IN THE PAPER CHROMATOGRAPHY OF NITROGENOUS COMPOUNDS

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INTRODUCTION

In previous papers we studied the use of chloranilic acid in the paper chromatography of inorganic compounds[1,2] and in the paper chromatography of the sodium salts of organic acids[3]. In this last case[3] we found that the sensitivity of the reagent towards the sodium salts of nitrogen-containing organic acids, such as nicotinic and *p*-aminobenzoic acids is higher than the values expected.

This was considered to be due to the reaction of chloranilic acid with the nitrogen of the pyridine nucleus, in the first case, and with the aromatic amine, in the second. This assumption was based on the work of BARRETO *et al.*, who used chloranilic acid for the assay of caffeine, theobromine and theophylline[4], coniine[5] and nicotinamide[6].

The present work was carried out in order to determine the sensitivity of chloranilic acid towards different types of nitrogenous compounds, in view of the possibility of applying it as a reagent in the paper chromatography of these substances.

MATERIALS AND METHODS

Reagent

Chloranilic acid was used as a 0.1 % (w/v) solution in amyl acetate. In previous work[1-3] ether was used as solvent, but in the present case we found that some of the spots were eluted by this solvent. Better results were obtained with amyl acetate.The reagent is stable when kept in a dark bottle.

Sample solutions

The samples were dissolved in water or in a suitable organic solvent, in concentrations of 0.1 % w/v (except for the alkaloids, when the concentration was 1 % w/v).

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Evaluation of sensitivity

As described previously[1], the sensitivity was first evaluated by spot tests on filter paper, followed by the determination of the minimum amount of material identifiable after the chromatographic separation.

Paper chromatography

The ascending technique was employed, using Macherey-Nagel No. 261 filter paper and a solvent system appropriate for the case under consideration.

Detection of the spots

The paper chromatograms were thoroughly dried in the oven at 120° and developed by dipping for 30 sec in an enamel tray containing the reagent (which was afterwards returned to the bottle). The chromatograms were then washed three times with amyl acetate, dried under the hood with the aid of an infra-red lamp and observed by ultra-violet light.

RESULTS

The sensitivity of chloranilic acid towards miscellaneous nitrogenous compounds separated by paper chromatography is shown in Table I, together with the R_F values found with each solvent system. It is worth noting that these values are not necessarily identical with the ones quoted in the literature, since the use of a different brand of filter paper can lead to results that differ, even when the same chromatographic technique is used.

TABLE I

SENSITIVITY (in μg) OF CHLORANILIC ACID TOWARDS VARIOUS TYPES OF NITROGENOUS COMPOUNDS SEPARATED BY PAPER CHROMATOGRAPHY

Compound	*Solvent system*	R_F	*Sensitivity*	*Ref.*
Glycine	BuOH–HAc–H_2O	0.30	2	
Alanine	(40:10:50)	0.43	2	7
Asparagine		0.22	2	
Nicotinic acid	BuOH–acetone–H_2O	0.62	6	
Nicotinamide	(45:5:50)	0.75	2	8
Isoniazid*		0.91	2	
Creatine	BuOH–EtOH–H_2O	0.18	6	9
Creatinine	(80:20:20)	0.40	6	
PABA**	BuOH–NH_4OH–H_2O	0.65	2	10
Sulphanilamide	(40:10:30)	0.60	2	
Caffeine	BuOH–HAc–H_2O	0.15	10	
Theophylline	(100:4:sat.)	0.18	10	11
Theobromine		0.05	50	

* Isonicotinic acid hydrazide.
** *p*-Aminobenzoic acid.

The sensitivity was expressed as the minimum amount (in μg) of the sample easily discernible on the developed chromatogram.

From the table it can be seen that chloranilic acid was tested as a reagent for various types of nitrogenous compounds, such as aliphatic and aromatic amines, amides, and guanidine, pyridine and purine derivatives. In most cases the sensitivity was 2 to 6 μg, but it was lower for the purine derivatives (10 to 50 μg).

DISCUSSION

The three characteristics of a good chromatographic reagent are (a) a high sensitivity, (b) a low specificity and (c) a simple developing technique. As regards the last two conditions, chloranilic acid is a very satisfactory reagent for nitrogenous compounds. Its sensitivity, however, is in general identical with that of the usual developers.

In the case of amino acids the sensitivity is ten to twenty times lower than that of ninhydrin, with the exception of asparagine, when it is of the same order[12]. For pyridine derivatives the sensitivity is very similar to that of cyanogen bromide[13], the usual reagent for these compounds. With respect to aromatic amines, the sensitivity is 2 to 10 times higher than that obtained with diazotized *p*-nitraniline[14].

Guanidine derivatives are usually identified by means of the Sakaguchi reaction, but this reaction gives no results with creatine and creatinine[15]. In this case the reaction with alkaline ferricyanide nitroprusside[16] is used, the results being comparable with those obtained with chloranilic acid.

For the purine alkaloids, the best reagent at the moment is the one suggested by HASSOM *et al.*[17], the sensitivity of which is higher than that of chloranilic acid with respect to caffeine (10 μg) but lower with respect to theobromine and theophylline (50 μg).

The results discussed in this paper show that chloranilic acid can be considered as a suitable reagent for the paper chromatography of nitrogenous compounds.

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SUMMARY

The authors studied the use of chloranilic acid as a reagent in the paper chromatography of various nitrogenous compounds, such as aromatic and aliphatic amines, amides, and guanidine, pyridine and purine derivatives.

The sensitivity was found to vary from 2 to 6 μg in most cases, and from 10 to 50 μg for the purine alkaloids.

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