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Note

Detection of aromatic sulphonic acids

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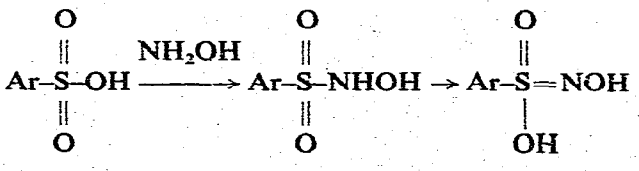
New demands are being made on analytical techniques in connection with the continuously increasing production of aromatic sulphonic acids, which are generally employed as intermediates in the manufacture of dyes. Paper and thin-layer chromatography have been very successful for the separation of mixtures of the various isomers. A number of detection techniques are available, the simplest of which involves observation under ultraviolet (UV) light of the acids alone or after spraying with pinacryptol yellow[1]. Acid–base indicators are also in common use. A less sensitive and non-specific technique consists of heating the chromatogram in a drying oven, whereupon the sulphonic acids cause blackening of the paper. Sometimes the sulphonic acids are fused with an alkali before chromatography and the resulting hydroxy derivatives are then identified[2,3].

None of the techniques that are utilized for qualitation is suitable for quantitation *in situ*. Heating of the chromatogram does not yield reproducible results, and acid–base indicators do not follow the concentration gradient in the spot. Measurement in the UV spectral region is complicated by the lack of suitable instruments for evaluation. Many workers have therefore eluted the spots from the chromatograms and then made a titrimetric analysis, either by measurement of the absorbance in UV light or by polarography using lead salts[4–9], etc.

The main difficulty encountered during detection in visible light is caused by the poor reactivity of the sulphonyl group, which is not convenient for densitometry. No problems are usually found when another functional group is also present.

A solution to this problem has been found in the reaction with hydroxylamine. If the chromatogram is sprayed with hydroxylamine, the sulphonyl group is converted into the sulphonohydroximic acid group, which then readily reacts with cupric ions to form a brown or brown-black colouration.



This colouration is very stable and follows the concentration gradient in the spot; consequently it is well suited for the densitometric evaluation of spots in the

visible spectral region. This method of detection has already been used for the identification of aromatic nitriles[10].

EXPERIMENTAL AND RESULTS

The detection of naphthalenesulphonic acids is based on spraying the chromatogram first with hydroxylamine and then, after drying, with a solution of cupric acetate. The spots which form are stable even after 1 year. The solution of hydroxylamine consisted of 5 g of hydroxylamine hydrochloride dissolved in 8 ml of distilled water; 10 g of anhydrous sodium carbonate were then added and the resulting mixture was dissolved in 200 ml of 96% ethanol. The solution was stirred, filtered and the filtrate was used for the spraying. This solution is stable for 2–3 days. The solution of cupric acetate was prepared by diluting a saturated aqueous solution of cupric acetate with water in a ratio of 1:3.

The dry chromatogram was sprayed with the dilute solution of cupric acetate after first spraying with hydroxylamine. The chromatogram was then allowed to dry for *ca.* 0.5 h and an intense colouration was obtained. The chromatogram was evaluated on a densitometer using a blue-green filter (maximum at 480 nm). The areas under the densitometric curves were compared with those obtained from a calibration graph. The amount of sample which was used was determined by the sensitivity to detection. The limits of detection of some sulphonic acids are given in Table I. 30, 50 and 70-μg amounts of 1% aqueous solutions were used to construct a calibration graph.

TABLE I

LIMITS OF DETECTION OF SULPHONIC ACIDS USING HYDROXYLAMINE AND CUPRIC ACETATE AS SPRAY REAGENTS

Acid	*Detection limit (μg)*
Toluene-*p*-sulphonic	10
Toluene-2,4-disulphonic	10
1,3,5-Trimethylbenzenemonosulphonic	—
m-Xylene-4-sulphonic	10
o-Xylene-3,5-disulphonic	30
p-Xylene-3,5-disulphonic	30
m-Xylene-4,6-disulphonic	20
Naphthalene-1-sulphonic	10
Naphthalene-2-sulphonic	10
Naphthalene-2,6-disulphonic	20
Naphthalene-1,5-disulphonic	20
Naphthalene-2,6-disulphonic	10
2-Methylnaphthalene-6-monosulphonic	40
Naphthalene-1,7-disulphonic	10
Naphthalene-1,3,7-trisulphonic	10
Naphthalene-1,3,5-trisulphonic	60
Naphthalene-1,3,5,7-tetrasulphonic	60
Anthracene-1-sulphonic	10
Anthracene-2-sulphonic	10
Anthracene-1,5-disulphonic	10
Anthracene-1,8-disulphonic	10
Anthracene-1,3,8-trisulphonic	40
Anthracene-1,3,5,8-tetrasulphonic	40

Separation of naphthalene-1-sulphonic acid from naphthalene-2-sulphonic acid

The separation of naphthalene-1-sulphonic acid from an excess of naphthalene-2-sulphonic acid is relatively difficult, but was achieved by using thin-layer chromatography (TLC). *tert.*-Butanol–absolute ethanol (1:1) was used as the mobile phase and Silufol UV-254 as the stationary phase. A 1% aqueous solution of the sample was placed on the chromatogram. When the atmospheric humidity was high, it was necessary to handle the plate and to place the sample on the plate as quickly as possible, since the separation of the two acids becomes impossible after deactivation of the silica layer. The plates were activated for 1 h at 120°. The detection was carried out as described using hydroxylamine and cupric acetate. The R_F values are given in Table II.

TABLE II

R_F VALUES ON Silufol UV-254 USING THE *tert.*-BUTANOL–ABSOLUTE ETHANOL (1:1) AS SOLVENT

Acid	R_F
Naphthalene-1-sulphonic	0.61
Naphthalene-2-sulphonic	0.00
Naphthalene-1,7-disulphonic	0.00
Naphthalene-2,6-disulphonic	0.00

DISCUSSION

It is obvious from the literature references that an aromatic sulphonic acid cannot be quantitated unless it contains another substituent that can be used for the detection, since densitometric evaluation cannot be made without an instrument which operates in the UV region. Therefore it seems that the described detection, utilizing the reaction of hydroxylamine with the sulphonyl group, can help in the determination of such acids.

The colouration (see Experimental section) is developed by spraying the chromatogram with a solution of a cupric salt. Spraying with a solution of a ferric salt was also examined, as it is known that hydroxamic acids, for example, give rise to intense colourations with ferric ions. However, the colouration is less intense and does not provide as great a contrast. The reaction with silver ions is also positive, but the chromatographic background cannot be removed by bathing the chromatogram. Other experiments showed that the reaction of sulphonohydroximic acid with cupric ions gives the best results. When the order of spraying was reversed, no colouration developed. During the investigation of the optimum colour-development procedure, it was found that the length of the period of treatment of the sulphonyl groups with hydroxylamine was less important than the requirement that the chromatogram be dried in the air between spraying with the two solutions. Using this procedure, only a light-blue background was obtained.

It is interesting that we failed to develop this colouration in solution, and that the reaction takes place only on paper or on a thin layer of silica. Salting-out chromatography cannot be used for the separation of aromatic sulphonic acids as the reaction does not take place in the presence of excess of salts.

As can be seen from Table I, the method of detection is of medium sensitivity. It is interesting that the monosulphonic acids can be detected most easily. It can be concluded that only the first sulphonyl group in the molecule is reactive; this also follows from the slopes of the calibration graphs for the individual acids. The stability of the developed colouration was also examined. One of the important advantages of this method of detection is the great stability of the colouration, even after many months. This results in a relatively low standard deviation (3.5%), compared with a number of other densitometric methods.

Despite the above advantages, a calibration graph must be constructed using the same chromatogram upon which the sample is to be analyzed, in order to minimize the error of the determination. It can be concluded that the present method of detection is an improvement an other methods of determination of aromatic sulphonic acids.

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