THE DETECTION OF SOME HALOGENATED PHENOLS AND NITROPHENOLS IN THIN-LAYER CHROMATOGRAPHY BY MEANS OF BROMINE

G. TADEMA AND P. H. BATELAAN
Laboratory of Organic Chemistry, State University, Croesestraat 79, Utrecht (The Netherlands)
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SUMMARY

A method is described for the detection of halogeno- and nitro-phenols in submicrogram quantities. These compounds are made visible by exposure of the developed thin layer plates to bromine vapour and subsequent spraying with an aqueous solution of potassium iodide or an ethanolic solution of fluorescein. This method is compared with other means of detection that are reported in the literature.

INTRODUCTION

There has been considerable interest in the use of thin-layer chromatography for the rapid separation of phenols. Many methods have been used for the detection of these substances after separation.

In their extensive investigations of the relationships between molecular structure and chromatographic behaviour of halogenated and alkyl-phenols, BARK AND GRAHAM[1] used alkaline potassium permanganate as a spray reagent. The usefulness of pi-electron acceptors for the detection of isomeric chlorophenols and their derivatives was determined by FISHBEIN[2], who found that of these reagents DDQ* was the most suitable.

VAN SUMERE and co-workers[3] employed diazotized *p*-nitroaniline to locate phenolic substances and coumarins and reviewed the use of analogous substances as chromogenic sprays.

Another detection agent was suggested by BARTON[4], who recommended a mixed solution of ferric chloride and α,α'-dipyridyl.

The halogens iodine and chlorine are also reported as non-specific reagents for locating phenols in trace amounts.

MILBORROW[5] showed that a combination of a fluorescent dye and iodine treatment resulted in a very sensitive method for the detection of many organic compounds on chromatoplates. An analogous method for detecting less than one microgram of substances of a phenolic nature has been suggested by EGOROVA AND KOLTUNOVSKAYA[6], while BROWN AND TURNER[7] have used iodine vapour for the estimation of phenolic steroids.

* DDQ reagent: 2% 4,5-dichloro-3,6-dioxo-1,4-cyclohexadiene-1,2-dicarbonitrile in benzene.

Chlorine/*o*-tolidine, a combination usually applied to detect NH-groups, also colours phenols, and was reported by WEBER AND LANGEMANN[8], who tested a series of phenols with these reagents; the sensitivities found were usually equal or better than those attained with standard tests for phenols.

We found that trace amounts of halogenated phenols can be detected also with bromine vapour.

After chromatography of phenols on a thin-layer plate, coated with silica gel, and subsequent exposure for a short time to bromine vapour in a closed tank, brown spots were observed in the places where phenols were present; these spots were rather vague and generally faded within minutes. If, however, this bromine-treated plate was then sprayed with a suitable reagent, the vague spots became clearly defined and remained visible. In this manner, many phenols could be detected in amounts of 0.1 μg or less.

When the chromatoplates used were coated with Silica Gel G containing a little amylopectin, an aqueous solution of potassium iodide or an ethanolic solution of fluorescein were found to be suitable sprays. Potassium iodide gave dark blue spots on a lighter background, while fluorescein, viewed under U.V. light, gave violet spots on a fluorescent background.

The usefulness of this method for detecting phenols was evaluated by comparison with existing procedures in the following manner:

(1) The sensitivities of eight methods of detection, not including the use of halogens, were estimated with *p*-chlorophenol as a test substance (Table I).

(2) The same was done for methods utilizing halogen (Table II).

(3) Seven of the best detection methods found in this way were subsequently tested with 17 other phenols (Table III).

(4) In order to obtain an idea to what extent the sensitivity of detection was affected by the developing solvent, thin layers to which *p*-chlorophenol had been applied were developed in five different solvent systems and subsequently subjected to the seven methods of detection. The sensitivities found in this way are given in Table IV.

(5) Since many phenols are known to give colours with chlorine/*o*-tolidine[8], we thought it also expedient to examine the effectiveness of the combinations bromine/*o*-tolidine and iodine/*o*-tolidine for locating phenols. The three combinations were tested with six phenols (Table V).

(6) The influence of the thin-layer material on the effectiveness of the detection by bromine was established by comparing chromatograms obtained on various absorbents (Table VII).

(7) Since the bromine–potassium iodide method proved to be the most useful, we investigated whether this method was suitable for semi-quantitative purposes (Table VIII).

(8) A preliminary survey of the usefulness of bromine for the detection of other compounds was also performed.

MATERIALS AND METHODS

Preparation of the plates

Thin-layer glass plates (5 × 20 cm) were coated with a Shandon spreader to a

thickness of 200 microns. The coating was performed according to one of the procedures outlined below:

(A) With Silica Gel G (Merck) and amylopectin (Koch Light Ltd.). 1.5 g of amylopectin and 15 ml of water were homogenized in a mortar. 28.5 g of Silica Gel G and 50 ml of water were added with stirring. The slurry obtained was applied to the plates. After drying in air for half an hour, the plates were heated in an oven at 110° for 2 h. Before use they were stored in a desiccator over silica gel for at least 3 days.

(B) As A, but amylopectin was omitted.

(C) As A, but instead of Silica Gel G (Merck) we used MN Silica Gel N, a product without gypsum from Macherey and Nagel.

(D) With MN Silica Gel S (Macherey and Nagel). 30 g of the powder was poured into 90 ml of boiling distilled water. The plates were coated without allowing the slurry to cool.

(E) With a mixture of cellulose and polyamide. The layers were prepared according to BARK AND GRAHAM[9] from cellulose powder MN 300 (Macherey and Nagel) and polyamide (Woelm).

(F) With a mixture of Silica Gel G and cellulose powder, preparation was according to VAN SUMERE *et al.*[3]. In order to prepare 20 plates 20 × 5 cm, 10 g of Silica Gel G and 10 g of cellulose powder (MN 300) were slurried with 80 ml of water and thoroughly homogenized. After spreading the thin layers were dried for about 6 h at room temperature.

(G) As F, only 1 g of amylopectin was added to 9.5 g of Silica Gel G and 9.5 g of cellulose powder.

Chromatography

The phenols were applied to the chromatograms dissolved in acetone. Aliquots of the solutions, containing 1.0, 0.50, 0.10 and 0.025* μg, respectively, were spotted at 1 cm intervals on the starting line of the chromatogram. The solutions were added in increments by means of a 10 μl Hamilton injector, so as to keep the spot size not greater than 3 mm in diameter.

After ascending chromatography (length of run 10 cm), the plates were dried with a stream of hot air from a hair dryer or by heating them in an oven at 110° for 10 min. They were then sprayed or subjected to other methods of detection. The minimum quantity of phenol, detectable in this way, was recorded in the appropriate table.

The spraying was performed with a Shandon laboratory spray gun. If necessary the chromatograms were viewed under a U.V. lamp (wave-length 254 mμ).

Sprays and other indicator methods

Detection methods not utilizing halogens (Table I)

General. The layers were prepared by procedure A and the developing solvent system was: benzene–ethanol 95:5 (v/v). After development the chromatograms were heated at 100° for 10 min before spraying with one of the following reagents:

* 10.0 mg of the substance was dissolved in 100 ml of acetone to give a solution containing 0.100 μg pro μl; a second solution containing 0.025 μg pro μl was prepared by dilution of the first (30 ml of acetone was added to 10 ml of this solution).

TABLE I

SENSITIVITY OF VARIOUS DETECTION METHODS FOR PHENOLS *not* UTILIZING HALOGENS

Thin layer: silica gel with amylopectin (procedure A, see text).
Developing solvent: benzene–ethanol (95:5, v/v).
Test substance: *p*-chlorophenol applied in amounts of 1.0, 0.50, 0.10, 0.025 μg.

No.	*Method of detection*	*Least detectable amount in μg*	*Colours*
1	Antimony trichloride	—*	
2	α,α′-Dipyridyl	—	
3	Folin phenol reagent	0.50	Bluish, very vague
4	Uranyl acetate	1.0	Dark under U.V.
5	DDQ	1.0	Dark-blue**
6	Potassium permanganate***	0.025	Yellow and brown
7	(a) Ferric chloride	—	
	(b) Potassium ferricyanide	—	
	(c) Ferric chloride + potassium ferricyanide	0.10	Green-blue
8	Diazotized *p*-nitroaniline****	0.50	Brownish-violet, rather weak

* — means 1 μg not detectable.
** Green after exposure to NH_3 vapour.
*** The reagent was employed in neutral and in basic solution. No difference in sensitivity was observed. The neutral solution gave better spots. Subsequent spraying with thymol blue or bromophenol blue did not effect appearance or permanency of the spots[12].
**** The spots produced by the reagents 3 and 8 attained their maximal intensity considerably later than those produced by the spray reagents 4, 5, 6 and 7.

(1) *Antimony trichloride*[10]. 34 g antimony trichloride in 100 ml of chloroform; after spraying the plates were heated for 5 min at 100°.

(2) *α,α′-Dipyridyl*[4]. A 0.5 % (w/v)* methanolic solution of α,α′-dipyridyl freshly mixed with 0.5 % aqueous ferric chloride.

(3) *Phenol reagent according to Folin and Ciocalteu (Merck)*. (a) Reagent diluted 1:4 with water; (b) a 2 *N* aqueous solution of sodium carbonate. The chromatogram was first sprayed with (a) followed by (b)[8].

(4) *Uranyl acetate*[11]. A 1 % aqueous solution of uranyl acetate; after spraying the plates were heated for 10 min, allowed to cool and viewed under U.V. light.

(5) *4,5-Dichloro-3,6-dioxo-1,4-cyclohexadiene-1,2-dicarbonitrile (DDQ)*[2]. A 2 % solution of DDQ in benzene, viewed before and after brief exposure to ammonia vapour. The substance used was a product of Schuchardt, München.

(6) *Potassium permanganate*. (a) Basic solution[1], 0.500 g of potassium permanganate and 0.515 g of sodium carbonate (anhydrous) in 100 ml of water; (b) neutral solution[12], 0.5 % aqueous solution of potassium permanganate.

(7) *Potassium ferricyanide–ferric chloride*[8,13]. Solution (a) ferric chloride, 3.6 % solution in water; (b) potassium ferricyanide, 1 % solution in water; (c) a mixture of (a) and (b) in equal volumes.

(8) *Diazotized p-nitroaniline*[3]. 5 ml of *p*-nitroaniline (0.5 % in 2 *N* HCl) and 0.5 ml sodium nitrite (5 % aqueous solution) were mixed at room temperature. Subsequently 15 ml of sodium acetate (20 % aqueous solution) was added to the mixture.

Before spraying with this reagent the plates were sprayed with 2 *N* sodium hydroxide.

* Henceforth the notation (w/v) will be omitted because it applies to all solutions prepared.

Detection methods utilizing halogens (Table II)

General. The layers were prepared by procedure A. The developing solvent was benzene–ethanol 95:5 (v/v).

The developed chromatograms were dried at 100° for 10 min. After cooling to room temperature they were placed in a chromatography tank filled with halogen vapour*.

When the tank contained bromine vapour they were removed after 3 sec, when it contained chlorine or iodine vapour this was done after 10 sec. To remove excess of halogen it was necessary to hold the plates in a stream of cold air (hair dryer), 1 min for iodine, 2 min for bromine or 3–5 min for chlorine, respectively. Finally they were sprayed with a suitable reagent.

Halogen vapours. The atmosphere of bromine was created by evaporation of an excess of the liquid in a closed tank at room temperature. The chlorine was prepared by the action of concentrated hydrochloric acid upon potassium permanganate. In some experiments chlorine obtained from a cylinder (dry or saturated with water vapour) was also used with essentially the same results. The iodine atmosphere was obtained in an analogous manner to that of bromine with iodine crystals.

Spray reagents. The following reagents were used:

(1) *Potassium iodide.* A 2 % solution of potassium iodide in water.

(2) *Fluorescein*[14]. A 0.2 % solution of fluorescein in ethanol; plates viewed under U.V. light.

(3) *Rhodamine B.* A 0.5 % solution of rhodamine B in ethanol; plates viewed under U.V. light.

(4) *Morin*[5]. A 0.06 % solution of morin in methanol; plates viewed under U.V. light.

TABLE II

SENSITIVITY OF VARIOUS DETECTION METHODS FOR PHENOLS UTILIZING HALOGENS

Thin layer: Silica Gel G with amylopectin (procedure A, see text).
Developing solvent: benzene–ethanol (95:5, v/v).
Test substance: *p*-chlorophenol applied in amounts of 1.0, 0.50, 0.10 and 0.025 μg.

*Method of detection**	*Least detectable amount in μg with*			*Colour of the spots*
	Chlorine	*Bromine*	*Iodine*	
Potassium iodide	0.10	0.025	0.025	Dark-blue
Fluorescein	0.50	0.025	0.025	Violet under U.V.
Rhodamine B	—	0.10	0.025	Violet under U.V.
Morin	0.50	0.10	0.10	Violet under U.V.

* We were unable to detect phenol on plates not treated with halogen with any of these spray reagents.

Comparison of several detection methods, using 18 phenols as test substances (Table III)

The layers were prepared as described under A. The developing solvent was *n*-hexane–ethanol 95:5 (v/v).

* It is essential that the plates are at room temperature; by exposing warm plates to halogen vapour spots of inferior quality are obtained. In the case of bromine even negative spots (lighter spots on a darker background) are formed.

TABLE III

SENSITIVITY OF PHENOL-DETECTION METHODS, TESTED ON 18 SUBSTRATES

Thin layer: Silica Gel G with amylopectin (procedure A, see text).
Developing solvent: *n*-hexane–ethanol (95:5, v/v).
The least detectable quantities are listed in the columns (in μg), colours are as described in Tables I and II.
The test substances were applied in amounts of 1.0, 0.50, 0.10 and 0.025 μg.

Name of the phenol	*Br_2/KI*	*Br_2/fluorescein*	*I_2/KI*	*I_2/rhodamine B*	*Cl_2/KI*	*$FeCl_3$ + K_3FeCN_6*	*$KMnO_4$*	*NH_3 vapour*
p-Chloro-	0.025	0.025	0.025	0.025	0.50	0.025	0.025	
o-Chloro-	0.025	0.10	0.025	0.10	0.10	0.025	0.10	
m-Chloro-	0.025	0.025	0.025	0.025	0.50	0.025	0.025	
2,3-Dichloro-	0.025	0.025	0.025	0.025	0.10	0.025	0.10	
2,4-Dichloro-	0.025	0.10	0.025	0.025	0.10	0.025	0.10	
2,5-Dichloro-	0.10	0.10	0.025	0.10	0.50	0.10	0.10	
2,6-Dichloro-	0.025	0.10	0.025	0.10	0.10	0.10	0.10	
3,4-Dichloro-	0.025	0.10	0.025	0.10	0.10	0.025	0.025	
3,5-Dichloro-	0.025	0.025	0.025	0.025	0.025	0.10	0.025	
2,4,5-Trichloro-	0.025	0.10	0.025	0.025	0.025	0.10	0.10	
2,4,6-Trichloro-	0.10	0.10	—*	0.10	1.0	0.10	0.10	
Pentachloro-	0.10	0.10	—	—	0.50	0.10	0.50	
p-Bromo-	0.025	0.025	0.025	0.025	0.50	0.10	0.025	
p-Fluoro-	0.025	0.025	0.025	0.025	1.0	0.025	0.025	
p-Nitro-	0.025	0.025	0.025	0.10	0.025	0.025	0.10	0.025
o-Nitro-	0.025	0.10	—	—	—	0.50	—	0.025
m-Nitro-	0.025	0.025	0.025	0.025	—	0.025	0.025	0.50
Phenol	0.025	0.025	0.025	0.025	0.025	0.025	0.10	

* — means 1 μg not detectable.

The methods of detection are the same as previously described, only the chromatoplates, on which the volatile substances phenol and *o*-chlorophenol had been applied, were not heated in an oven, but dried with a stream of warm air for 3 to 5 min. The phenols used as test substances were chromatographically pure.

Influence of the developing solvent system on the sensitivity of the detection method (Table IV)

The layers were prepared as described under A. The developing solvent systems were:

(1) Benzene–ethanol, 95:5 (v/v);
(2) *n*-Hexane–ethanol, 95:5 (v/v);
(3) Benzene–acetone, 97.5:2.5 (v/v)[2];
(4) Diethyl ether–*n*-hexane, 1:1 (v/v);
(5) Benzene–acetic acid, 5:1 (v/v)[2].

The methods of detection are the same as previously described.

Detection method utilizing halogen/o-tolidine (Table V)

The layers were prepared by procedure A and the developing solvent was *n*-hexane–ethanol, 95:5 (v/v).

The thin-layer plates were treated as described previously (with reference to Table II), only the times of exposure to halogen and blowing of air were altered.

TABLE IV

INFLUENCE OF THE DEVELOPING SOLVENT SYSTEM ON THE SENSITIVITY OF THE DETECTION METHOD

Thin layer: Silica Gel G with amylopectin (procedure A, see text).
Test substance: *p*-chlorophenol applied in amounts of 1.0, 0.50, 0.10 and 0.025 μg.
The least detectable quantities (in μg) are listed in the columns.

Solvent systems	*Cl_2/KI*	*I_2/KI*	*I_2/rhodamine B*	*Br_2/KI*	*Br_2/fluorescein*	*$FeCl_3$ + K_3FeCN_6*	*$KMnO_4$*
Benzene–ethanol	0.10	0.025	0.025	0.025	0.025	0.10	0.025
n-Hexane–ethanol	0.10	0.025	0.025	0.025	0.025	0.025	0.025
Benzene–acetone	0.10	0.025	0.025	0.025	0.10	0.10	0.10
Ether–*n*-hexane	0.10	0.10	0.025	0.025	0.10	0.10	0.10
Benzene–acetic acid*	0.10	0.10	—	0.10	—	0.10	0.10

* The spots obtained in this solvent are the least distinct of all.

TABLE V

DETECTION METHODS UTILIZING HALOGEN/*o*-TOLIDINE/POTASSIUM IODIDE

Thin-layer: Silica Gel G with amylopectin (procedure A, see text).
Developing solvent: *n*-hexane–ethanol (95:5, v/v).
Test substance: *p*-chlorophenol applied in amounts of 1.0, 0.50, 0.10 and 0.025 μg.
The least detectable quantities (in μg) are listed in the columns.

No.	*Name of the substrate*	*o-Tolidine combined with*		
		Chlorine	*Bromine*	*Iodine*
1	*p*-Chlorophenol	0.10	0.025	0.025
2	*m*-Chlorophenol	0.10	0.025	0.025
3	3,5-Dichlorophenol	0.025	0.025	0.025
4	2,4,5-Trichlorophenol	0.10	0.025	0.10
5	Pentachlorophenol	0.10	0.10	—
6	*p*-Nitrophenol	0.10	0.025	0.025

After some experimental work we found that the exposure times shown in Table VI were optimal:

TABLE VI

OPTIMAL TIMES OF EXPOSURE TO HALOGEN AND AIR BLOWING, USING HALOGEN/*o*-TOLIDINE/POTASSIUM IODIDE AS DETECTION REAGENTS

Halogen	*Exposure to halogen (sec)*	*Air blowing (min)*
I_2	10	2
Br_2	3	2
Cl_2	10	5

A cold saturated solution of *o*-tolidine in 2 % acetic acid and a 2 % aqueous solution of potassium iodide[8] were subsequently used as sprays.

Effect of varying the adsorbent layer (Table VII)

Thin layers, coated according to one of the procedures described for the prep-

TABLE VII

INFLUENCE OF ADSORBENT LAYER ON SENSITIVITY OF DETECTION METHOD

Developing solvent: *n*-hexane–ethanol (95:5, v/v).
Test substance: *p*-chlorophenol (0.025 μg applied).
General procedure: in bromine vapour for 3 sec, in stream of cold air for 2 min.
Key to symbols etc.: ap = amylopectin; cel = cellulose; g = gypsum; s = starch; sg = silica gel. + + + + + = excellent; + + + + = good; + + + = fairly good; + + = moderate; + = inferior.

Procedure for preparation of the layers (see text)	*Composition of the layers*	*Stability of the layers*	*Sensitivity and distinctness of the spots located with*	
			*Bromine/fluorescein***	*Bromine/potassium iodide*
A	sg, ap, g	+ + + +	+ + + +	+ + + +
B	sg, g	+	+ + + +*	—
C	sg, ap	+	+ + +	+ + +
D	sg, s	+ +	+ + + +	+ + + + + *
E	pa, cel	+ + + + +	+ +	—
F	cel, sg	+ + +	+ + + +**	—
G	cel, sg, ap	+ + + +	+ + + +	+ + + +

* In a stream of cold air for 1 min.
** In bromine vapour for 1 sec, in stream of cold air for 15 sec.
*** 0.025 μg can be detected on all substrates (except on E); spots with the least background are produced on D; the spots are somewhat less distinct than on the other substrates on C. More than one microgram is necessary for detection on E (cellulose + polyamide).

aration of the thin-layer plates, were used for the chromatography of the test substance *p*-chlorophenol, with *n*-hexane–ethanol (95:5, v/v) as developing solvent. They were exposed to bromine vapour and subsequently sprayed with solutions of potassium iodide or fluorescein.

With some layers, better results were obtained when the periods of exposure to bromine vapour and to a stream of cold air were shortened.

Semiquantitative determination of phenol (Table VIII)

The layers were prepared by procedure A. The developing solvent systems were: *n*-hexane–ethanol (95:5, v/v) and benzene–ethanol (95:5, v/v). Test substances

TABLE VIII

SEMIQUANTITATIVE DETERMINATION OF PHENOL

Thin layer: Silica Gel G with amylopectin (procedure A, see text).
Test substances: *p*-chlorophenol and 3,5-dichlorophenol.
Developing solvent systems: *n*-hexane–ethanol (95:5, v/v) and benzene–ethanol (95:5, v/v).
The ranges of quantities applied are listed in column A.
The least detectable difference between two quantities of phenol is recorded in column B.
The results obtained with both test substances and in both developing solvents are identical.

A in μg	*B in μg*
4–2	1
2–1	0.5
1–0.25	0.25
0.25–0.10	0.10
0.10–0.025	0.025

used were *p*-chlorophenol and 3,5-dichlorophenol and detection was with bromine–potassium iodide as described.

In order to find out the least difference between two quantities of *p*-chlorophenol that still could be detected, a series of increasing quantities was applied to a thin layer and subjected to chromatography. The spots obtained were compared with regard to their intensity of colour, extensiveness and appearance*.

The smallest detectable difference between two quantities of *p*-chlorophenol can be determined by observation of the spot intensity in a series of concentrations, for instance the following: 4, 3, 2, 1.5, 1.0, 0.75, 0.50, 0.25, 0.10, 0.05 and 0.025 μg.

The experiments with both test compounds and with the two developing solvents, led to identical results (Table VIII).

RESULTS

The detection agents potassium ferricyanide–ferric chloride[13] and potassium permanganate are the most sensitive of the sprays listed in Table I. Nearly all the phenols investigated could be detected in amounts of 0.1 μg or less.

The methods utilizing halogen are equivalent or in some cases superior to the two just mentioned. The combinations of bromine–potassium iodide and bromine–fluorescein were very effective; most of the phenols investigated could be detected in quantities of 0.025 μg and the spots retained their colour and sharpness for weeks. Detection with potassium iodide was the most sensitive; the spots obtained are visible in daylight and this method can be performed rapidly and with simple reagents**.

Though the methods utilizing chlorine are fairly effective, in general they were exceeded in sensitivity, rapidity of performance and lack of background on the plates by the methods using iodine or bromine. The results obtained with iodine and bromine were almost equal; some substances, however, not detectable with iodine, can be located with bromine (see Table III).

The order of sensitivity of the four spray reagents used in combination with halogens (Table II) is as follows: potassium iodide > fluorescein > rhodamine B > morin. The order of distinctness of the spots is the same. Regarding the permanence of the spots, those obtained with potassium iodide last for weeks, while those produced by rhodamine B, fluorescein or morin remain visible under a U.V. lamp for some days. Other spray reagents were also tested for their usefulness in detecting phenols on plates treated with bromine vapour, *viz.* dithiazone, fuchsin, phenol red and methyl red[14]. All proved to be inferior to those listed in Table II.

When a developed plate is held in iodine vapour, the phenols are visible as brown spots which are rather vague. Fixation of the spots can be obtained by spraying

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* When more than a certain quantity of phenol is applied, a light-coloured nucleus is formed in the blue spot on the chromatogram. This phenomenon can also be used for the estimation of the amount of phenol.

** While we were completing this paper it was brought to our attention that POGACAR *et al.*[15] had used only bromine as a reagent for the detection of several compounds, some of which had a phenolic character. Comparison of this method with that described in our paper proved that the latter is much better for the detection and estimation of sub-microgram amounts of the compounds listed in Table III.

with a solution of potassium iodide. The resulting dark-blue spots last for weeks, and the sensitivity of the detection method is moderately enhanced*.

With other sprays the visibility of the spots is somewhat improved, but the sensitivity of the iodine method is hardly altered.

With regard to the development of the chromatograms, the solvent system *n*-hexane–ethanol (95:5, v/v) proved to be the best. The systems benzene–ethanol (95:5, v/v) and ether–*n*-hexane (1:1, v/v) are almost as good, while benzene–acetic acid (5:1, v/v) gave less satisfactory results when about 1 μg or less of phenol is applied. With quantities of about 3 μg this system can be used as well.

With respect to the detection of the nitrophenols, it was noted that *p*- and *o*-nitrophenol are visible as such in daylight; thus quantities of 0.1 μg can be seen as yellow spots on chromatograms. The colour and visibility of these spots can be enhanced by exposing the plates to ammonia vapour, after which amounts down to 0.025 μg can be seen. Other detection methods give no better results, only bromine–fluorescein or bromine–potassium iodide gave some improvement. The behaviour of *m*-nitrophenol is comparable to that of the halophenols.

Of all the phenols the *ortho* substituted ones are the most difficult to detect, especially *o*-nitrophenol, 2,4,6-trichlorophenol and pentachlorophenol. It is of interest that this phenomenon is apparent both for the methods utilizing halogen and for those utilizing potassium permanganate or potassium ferricyanide/ferric chloride (Table III).

The compounds listed in Table III are readily detectable with bromine–*o*-tolidine in amounts of 1 μg; only for *o*-chlorophenol, *o*-nitrophenol and 2,6-dichlorophenol 3 μg is needed. When spots obtained thus are sprayed with a solution of potassium iodide, the colour of the spots is intensified, but the sensitivity of the method is hardly enhanced, which is in accordance with the findings of WEBER AND LANGEMANN[8]. In combination with *o*-tolidine iodine and bromine generally give better results than chlorine (Table V). When the plates, after exposure to halogen, are subsequently sprayed with potassium iodide and with *o*-tolidine, the latter reagent has very little effect on the appearance of the spots. For that reason the use of this potential carcinogen was avoided.

The sensitivities attained by the bromine method on thin layers of different composition are very satisfying in general; in addition, the spots obtained are distinct. When a potassium iodide solution is used as a spray, it is of course essential that the layers contain amylopectin or starch; amounts of 0.1 to 0.025 μg of *p*-chlorophenol can thus be detected.

Analogous results can be obtained with a fluorescein solution, both on layers containing amylopectin and on substrates without this substance.

The stability of the layers (as listed in Table VII) is an important factor; layers with too low a stability are in danger of being destroyed by spraying and even more so by the air blowing from the hair dryer. Taking into account both sensitivity and stability, in our opinion the best layers are those prepared according to proce-

* When a solution of potassium iodide is sprayed on the spots where iodine has been adsorbed, the halogen goes into solution and thus penetrates the thin layer where it combines with the amylopectin to form the blue compound. By spraying the chromatograms with distilled water, in which iodine is sparingly soluble, only slight fixation of the spots is attained.

dure A (silica gel with amylopectin and gypsum), D (silica gel with starch) and G (silica gel and cellulose with amylopectin).

The sensitivity of the bromine–fluorescein method is lower when the plates are coated with cellulose and polyamide (procedure E). Although quite good results can be obtained with some compounds, on the whole more than 1 μg has to be applied in order to get a clear spot.

The appearance of a spot produced with bromine or iodine is more dependent on the quantity of the phenol applied than is the case with spots obtained by other methods.

As can be concluded from Table VIII, the bromine–potassium iodide method can be used to estimate the concentration of phenols in a solution when quantities of these compounds ranging from about 0.1 μg up to 4 μg are applied to the thin layer.

We have applied the method to the estimation of small quantities of phenols present as impurities in samples of phenyl esters. In some cases it was possible to detect 0.1 μg of phenol in 100 μg samples of phenyl ester applied to a thin layer. In a subsequent publication this and other applications of the method will be reported in more detail.

In preliminary experiments we found that the bromine–potassium iodide method is also suitable for the detection of substances such as resorcinol, phloroglucinol, catechol, pyrogallol, *p*-aminophenol, thiophenol and of phenolic esters of N-protected amino acids.

As was anticipated by analogy to the findings of WEBER AND LANGEMANN[8], many compounds containing NH_2 or NH groups also gave coloured spots, *e.g.* aniline, acetamide, phenacetin and the amino acids tryptophan, tyrosine and asparagine. Moreover some peptides and derivatives of peptides such as are used in peptide synthesis can be located with bromine–potassium iodide.

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