

of the iodine indicator we could thus calculate with reasonable accuracy the composition of mixtures of I and II.

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A method for quantitative thin-layer chromatography using the flask combustion method

Generally, an extraction or a densitometric method is used for quantitative thin-layer chromatography. Previously, we reported another useful method whereby a gas chromatographic technique of elementary analysis is used for the determination of organic substances containing nitrogen¹. On the other hand SOEP^{2,3} has reported the determination of fluorine on paper chromatograms by means of the flask combustion method. In the present paper, we describe a convenient method for the determination of organic substances containing halogens on thin-layer chromatograms by the flask combustion method^{4,5}.

Apparatus and procedure

The combustion flask consists of a 500 ml Erlenmeyer flask and a sample holder or an electrode^{4,5}. Chromatoplates (5 × 20 cm) were layered with Kiesel Gel H (Merck AG, Darmstadt), Wako Gel B-10 (Wako Chem., Osaka), Aluminium Oxide G (Merck), Cellulose TLC (Serva, Heidelberg) or Silica Gel H-F, in the usual manner (0.2-0.3 mm thickness). Silica Gel H-F was prepared by mixing 80 ml of 0.003 % aqueous uranine (Fluoresceine sodium) solution with 32 g of Kiesel Gel H.

Samples (ca. 400 μg) dissolved in ethyl acetate, benzene or methanol were

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applied on various chromatoplates with a Hamilton syringe and developed by the ascending method (*ca.* 12 cm) using suitable solvent systems. The spots were detected by U.V. light or spraying of 0.1 % ethanol solution of methyl yellow, and then scraped off from the plate. The gel powder (*ca.* 80 mg) was scraped off and wrapped in a suboctuple of a filter paper (Toyo-Roshi No. 7; 11 cm diameter); following which it was burnt in a combustion flask containing an aqueous adsorbent.

In the case of the determination of chlorine or bromine, 5 ml of water containing 0.5 ml of 30 % hydrogen peroxide was used as an absorbent. After the combustion the solution was diluted with 20 ml of ethanol, and titrated with 0.005 *N* mercuric perchlorate solution* (indicator: diphenylcarbazone-urea (1:25)).

TABLE I

BLANK VALUES OF THE FILTER PAPER AND ADSORBENTS^a

Substance	Titrated ml	St. dev. ml	Cl ^b μg	Br ^b μg	n
Toyo-roshi No. 7	0.064	0.0097	11.3	25.5	25
Kieselgel H	0.108	0.014	19.2	43.3	10
Wako gel B-10	0.180	0.022	31.9	71.7	8
Aluminium oxide G	0.108	0.014	19.2	43.3	9
Cellulose TLC	0.208	—	36.9	83.1	2

^a Sample: Paper, 11 cm (diam.) × 1/8; Adsorbent, 0.25 mm × 2 cm². Titrant: 0.005 *N* Hg(ClO₄)₂. Values of the adsorbents include the blank value of the filter paper.

^b Calculated values from titration values as chlorine or bromine.

TABLE II

DETERMINATION OF SAMPLES SPOTTED ON TLC PLATES

Adsorbent: Kieselgel H 0.25 mm × (2~3)cm².

Sample	Wt. μg	Halogen		Recovery %	St. dev. %	n
		Calc. μg	Found μg			
<i>m</i> -Chloroacetanilide	499.5	Cl 104.4	104.1	99.7	3.0	5
2,4-Dichlorophenoxyacetic acid	335.0	Cl 107.5	105.7	98.3	2.5	7
<i>γ</i> -BHC	409.8	Cl 299.7	291.0	99.1	0.5	7
Tetrachlorophthalic anhydride	414.0	Cl 205.3	200.6	97.4	1.4	5
<i>p</i> -Bromoacetanilide	420.5	Br 156.8	151.9	96.9	0.6	5
<i>o</i> -Iodobenzoic acid	401.2	I 210.8	107.7	98.6	0.4	4
5-Chloro-7-iodo-8-quinolinol	247.6	I 102.9	102.8	99.9	0.6	4

In the case of the determination of iodine, 5 ml of water containing three drops of 30 % sodium hydroxide solution was used. After absorption, the solution was diluted with 15 ml of water, 5 ml of the bromine solution** were added and it was allowed to stand for a few minutes. Then a 90 % solution of formic acid was added to the solution

* 0.005 *N* Mercuric perchlorate solution was prepared by dissolving 270 mg of mercuric oxide in 0.5 ml of 70 % perchloric acid solution and diluting with water to 500 ml. This solution was standardized using sodium chloride.

** Bromine solution was prepared by dissolving 1 ml of bromine and 20 g of potassium acetate in 250 ml of acetic acid.

till the color of the solution disappeared. After standing for 5 min the solution was titrated with 0.01 *N* sodium thiosulfate solution according to the usual methods of iodometry.

Results and discussion

Blank values of Cl and Br in the filter paper and the various adsorbents for TLC are shown in Table I. Subsequent to these results, Kiesel Gel H was used mainly as the adsorbent. The use of the outside papers of a filter bundle was not recommended because of high blank value of both halogens. Blank values of iodine in the filter paper and all the adsorbents were within 0.05 ml.

TABLE III
DETERMINATION OF SAMPLES DEVELOPED ON TLC PLATES

Sample	Wt. μg	Solvent ^a	R _F	Halogen		Recovery ^b %
				Calc. μg	Found μg	
<i>m</i> -Chloroacetanilide	393.5	H:E (1:1)	0.3	82.3	81.2	97.7
γ-BHC	422.3	B:P.E. (1:1)	0.8	308.8	305.3	98.9
2,4-Dichlorophenoxyacetic acid	419.4	B:A (95:5)	0.5	134.5	130.3	96.8
2,4-Dinitrochlorobenzene	405.4	H:B (95:5)	0.4	71.0	68.4	96.6
<i>p</i> -Bromoacetanilide	411.4	E	0.6	153.6	149.4	97.4
<i>o</i> -Bromobenzoic acid	403.8	B:A (95:5)	0.5	160.5	156.8	97.6
<i>o</i> -Iodobenzoic acid	415.3	B:A (95:5)	0.5	212.5	210.4	99.0
Iodoacetamide	389.3	E	0.4	267.1	260.3	97.4

^a H = *n*-hexane; E = ether; B = benzene; P.E. = pet. ether (b.p. 30–60°); A = acetic acid.

^b Standard deviations were within 3%.

TABLE IV
SIMULTANEOUS DETERMINATION OF MIXED SAMPLES

Mixed sample	Weight		Solvent ^a	R _F	Halogen		Calc. sample ratio %		
	μg	%			Calc. μg	Found μg			
γ-BHC	513.7	83.5	H:E (1:1)	0.8	375.5	373.4	83.0		
<i>m</i> -Chloroacetanilide		16.5		0.3			21.2	21.2	16.5
γ-BHC	102.7	16.8	H:E (1:1)	0.8	75.1	73.4	16.4		
<i>m</i> -Chloroacetanilide		83.2		0.3			106.0	106.4	83.3
<i>m</i> -Chloroacetanilide	406.0	66.9	B:E (1:1)	0.4	84.8	84.5	66.5		
<i>m</i> -Chloroaniline		16.5		0.8			28.0	—	— ^b
Acetanilide		16.6		0.4			0	—	—
<i>o</i> -Bromobenzoic acid	417.0	50.4	B:A (98:2)	0.3	165.8	169.2	51.4		
Ethyl- <i>o</i> -bromobenzoate		49.6		0.9			143.4	—	— ^b
<i>o</i> -Iodobenzoic acid	203.8	33.5	B:A (95:5)	0.3	104.3	105.5	33.9		
Iodoacetamide		66.5		0.1			277.4	279.4	67.0

^a H = *n*-Hexane; E = ether; B = benzene; A = acetic acid.

^b Volatile samples were not determined.

Table II summarizes the assayed results of samples applied to the silica gel plates without development. It should be noted that the area of the layer scraped off was limited to 2-3 cm² maximum and the capacity of a combustion flask was 500 ml minimum, otherwise the combustion was incomplete.

Table III shows the assayed results of samples developed on silica gel plates.

Table IV shows the results of the simultaneous determination of mixed samples by TLC.

The convenient technique described above is useful for the determination of a compound containing halogens which has a low vapor pressure and/or is thermally unstable, and consequently cannot be determined by gas chromatography. In particular, the method is exceedingly effective for the determination of a crude compound which contains various unknown impurities and is difficult to determine by other methods.

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