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A method for quantitative thin-layer chromatography using organic ultramicro carbon analysis

Although quantitative techniques for the thin-layer chromatography of organic nitrogen compounds using an elementary nitrogen analysis and of organic halogen compounds using the flask combustion method have been established in our laboratory[1,2], these methods can only be applied to a few compounds containing nitrogen or halogens. If such TLC techniques can be combined with an elementary carbon analysis, a wider application may be expected. While studying quantitative TLC, we found that the use of less polar, more volatile solvents for development followed by their removal from the layer under adequate drying conditions after concentration of the adsorbing components by the method of DE DEYNE AND VETTERS[3] allowed the determination of each component in the spots by elementary carbon analysis. The procedures and results with this method are described.

The apparatus described by KONAKA AND TERABE[1] was modified as follows: a furnace was separated into two parts, a sample combustion furnace (kept at 950–1000°) and a cupric oxide heating furnace (kept at 800°). A reduced copper heating furnace was kept at 550°. Plates (5 × 20 cm) were coated with Silica Gel H (Merck AG, Darmstadt) containing 3 % of Fluorescent powder P 22-G 1 (Dainippon Painting Material Co., Kanagawa, Japan) to a thickness of 0.2–0.3 mm by usual method.

A solution of a sample in ethyl acetate, benzene or acetone is applied to the plate, which is developed by a mixture of volatile solvents such as ether, petroleum ether, methyl acetate, benzene, acetone or chloroform. The plate is viewed under U.V. light, and each component in the adsorbing spots is concentrated in a small area by the horizontal elution technique of DE DEYNE AND VETTERS[3] using acetone. Then the
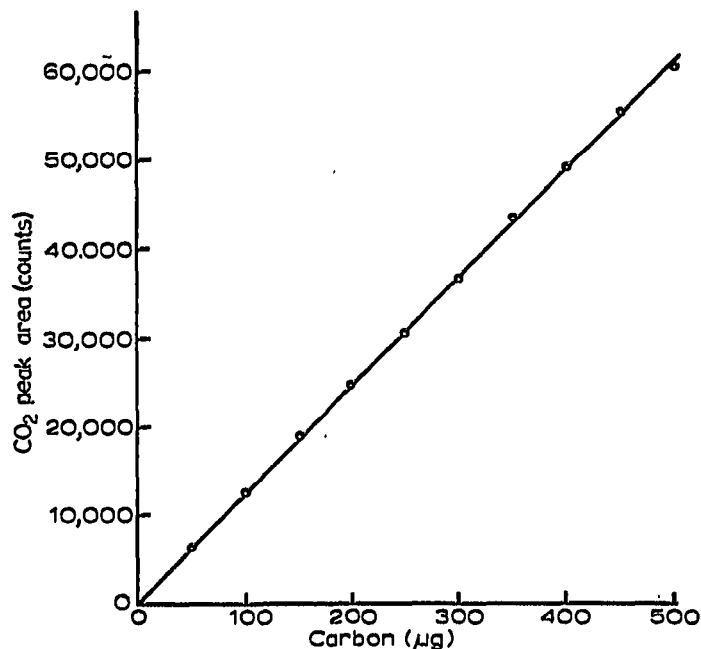

Fig. 1. Relationship between peak area of CO_2 and amount of carbon.

plate is dried under a stream of air at 70° for 5 min or under 2 mm Hg at 60–70° for 2 min followed by cooling to room temperature under helium or nitrogen. Each adsorbing spot thus obtained is scraped off into a platinum boat and is burned under helium gas containing 2 % oxygen. The amount of each component is estimated from the peak area of carbon dioxide in the gas chromatogram measured by an electronic integrator with blank correction.

The calibration curve indicated a good linear relationship between the amount of carbon and peak area of carbon dioxide as shown in Fig. 1. Table I shows the results obtained with pure samples developed on the plate. A test of the reliability of

TABLE I

DETERMINATION OF PURE SAMPLES DEVELOPED ON THE SILICA GEL PLATES

Sample	Weight (μg)	Solvent[a]		R_F value	Carbon		Recovery (%)
					Calc. (μg)	Found (μg)	
Benzoin	201.3	B–A	(15:1)	0.6	159.5	150.0	94.0
($C_{14}H_{12}O_2$)	204.4				161.9	159.3	98.4
Phenothiazine	293.3	PE–E	(2:1)	0.6	212.1	219.5	103.5
($C_{12}H_9NS$)						210.2	99.1
Phenoxazine	197.7	PE–E	(2:1)	0.6	155.7	159.6	102.5
($C_{12}H_9NO$)						152.4	97.9
p-Toluenesulfonamide	200.0	PE–E	(1:3)	0.6	98.2	96.2	98.0
($C_7H_9NO_2S$)						94.7	96.4
p-Nitroaniline	203.0	PE–E	(1:5)	0.5	105.9	110.9	104.7
($C_6H_6N_2O_2$)						106.7	100.7
Acetanilide	197.9	B–A	(2:1)	0.5	140.7	141.9	100.8
(C_8H_9NO)						133.0	94.5
Sulfanilamide	199.2	B–E	(1:1)	0.6	83.4	81.2	97.4
($C_6H_8N_2O_2S$)						77.6	93.4

[a] PE = petr. ether (boiling range 30–40°), E = ether, A = acetone, B = benzene.

TABLE II

SIMULTANEOUS DETERMINATION OF MIXED SAMPLES

Mixed sample	Weight		Solvent[a]		R_F value	Carbon		Calc. sample ratio (%)
	(μg)	(%)				Calc. (μg)	Found (μg)	
p-Nitroaniline	196.9	48.7	E–PE	(5:1)	0.8	102.7	88.1	41.7
Acetanilide	207.6	51.3			0.5	147.6	129.5	45.0
p-Nitroaniline	169.9	49.2	PE–C	(1:1)	0.5	102.7	101.6	48.7
Benzoine	203.0	50.8			0.1	160.8	148.0	46.8
Sulfanilamide	199.9	33.3	E–PE	(4:1)	0.2	83.6	109.1	43.4
p-Toluenesulfonamide	200.8	33.4			0.5	98.6	96.5	32.7
Benzoine	199.8	33.3			0.9	158.3	126.9	26.7
Sulfanilamide	98.4	24.9			0.2	41.2	43.8	26.5
Acetanilide	102.2	25.8	MA–C	(1:3)	0.4	72.7	71.2	25.2
p-Nitroaniline	96.9	24.5			0.6	50.6	46.6	22.5
Benzoine	98.4	24.8			0.8	77.9	74.2	23.6

[a] E = ether, C = chloroform, MA = methylacetate, PE = petr. ether (boiling range 30–60°).

TABLE III

DETERMINATION OF MINOR COMPONENTS IN MIXED SAMPLES

Sample	Weight		Solvent[a]	R_F value	Carbon		Calc. sample ratio (%)
	(µg)	(%)			Calc. (µg)	Found (µg)	
Benzoine	117.5	5.8	B–A (15:1)	0.6	93.1	88.3	5.5
p-Nitroaniline	1900.0	94.2		0.3	—	—	—
p-Toluenesulfonamide	120.0	5.9	PE–E (1:3)	0.6	58.9	54.2	5.5
Sulfanilamide	1900.0	94.1		0.2	—	—	—
Acetanilide	40.6	2.0	B–A (2:1)	0.5	28.8	26.1	1.8
Antipyrine	1950.0	98.0		0.3	—	—	—
p-Nitroaniline	121.7	6.0	B–E (2:1)	0.5	86.5	79.7	5.6
Acetanilide	1900.0	94.0		0.3	—	—	—
p-Nitroaniline	81.3	4.1	PE–E (1:5)	0.5	42.4	44.2	4.3
Acetanilide	1900.0	95.9		0.2	—	—	—
p-Bromoacetanilide	40.0	2.0	B–A (3:1)	0.5	18.0	17.8	2.0
Antipyrine	1950.0	98.0		0.2	—	—	—
p-Bromoacetanilide	160.6	8.0	B–A (3:1)	0.5	72.1	66.5	7.4
Antipyrine	1850.0	92.0		0.2	—	—	—

[a] PE = petr. ether (boiling range 30–60°), E = ether, A = acetone, B = benzene.

this method, as applied to the separation and recovery of components from a mixed component system, is shown in Table II. It was indispensable to reduce the blank value which played an important part in the accuracy of the recovery data that the organic solvent for development containing large amount of carbon was removed as completely as possible from the plate. For this purpose we used the volatile solvents and concentrated the component in the adsorbent by the method of DE DEYNE AND VETTERS[3]. These procedures reduced the blank value up to 10–30 µg of carbon. A further problem may arise from contamination by carbon dioxide in air. This could be excluded to some extent by the use of an atmosphere of helium or nitrogen. Table III demonstrates the effectiveness of this method as applied to a minor component in a mixed sample. These results show that this method also conveniently may be applied to the determination of a minor component in a mixed component system, for example known impurities in drugs.

The use of volatile solvents in our TLC techniques previously reported may permit the determination of many organic compounds by means of elementary carbon analysis which is difficult by other methods.

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