same field, using the same application technique, crop, and management.

Carbaryl, as expected, was less persistent in the soil than carbofuran: the times for 95% disappearance were 135 and 400 days, respectively. Although carbofuran exhibited a short lag period after band application, its disappearance conformed in general to a first-order reaction. This was not true for carbaryl, as shown above. Most interestingly, both pesticides degraded much faster in certain small areas of the watershed than in the remainder of the field, but the areas were not the same for the two pesticides. With carbofuran, the rapid degradation was associated with moisture content and pH of the ambient soil; no such relationship was observed with carbaryl.

The different loss of the two pesticides in runoff water reflects differences in their physicochemical properties: carbaryl is less water soluble than carbofuran (99 and 250 ppm, respectively) and is adsorbed to soil surfaces to a greater extent. The difference in adsorption was established in laboratory measurements of adsorption isotherms, in which we found Freundlich k values of 2.20 for carbaryl and 0.51 for carbofuran in two experiments run under identical conditions. Although runoff from the watershed was roughly four times as much in 1973 as in 1971, the water contained only one-third as much pesticide. The observed pesticide distribution coefficients between sediments and water (K_d) were 0.06 and 0.33 in the carbofuran and carbaryl experiments, respectively. The concentration of pesticide on sediments was below 1 mg/kg of sediment in both cases and the gross transport of both pesticides on eroded soil was consequently very low.

Because carbofuran is readily taken up through plant roots and translocated into aerial portions of the plants, it is an effective systemic insecticide. Carbaryl, on the other hand, has only slight systemic action. As evidence of this, no residues of carbaryl or related compounds were found in harvested corn samples in these experiments, whereas carbofuran and its metabolites were readily detected in the 1971 corn plants.

ACKNOWLEDGMENT

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Determination of Diquat and Paraquat Residues in Soil by Gas Chromatography

Shahamat U. Khan

An analytical method is described for the gas chromatographic determination of diquat and paraquat residues in soil. The method involves extraction of the soil with 18 N H_2SO_4, followed by catalytic hydrogenation of the acid extract, and determination by gas chromatography. Recoveries of these two herbicides added to soils at 0.1- and 0.05-ppm levels were between 84 and 95%. The lower limit of sensitivity for this method is approximately 0.01 ppm. The method has been used for the determination of field applied diquat and paraquat in three different types of soil. Hydrogenation of acid extracts from organic soil required more catalyst per gram of soil than from mineral soil. The pH of the soil extract from 1 to 9 had no effect on the recovery of field applied herbicide residues. The capabilities of various columns, an alkali flame ionization detector (AFID), and a flame ionization detector (FID) have also been compared for the analyses of diquat and paraquat.

The herbicides diquat (1,1′-ethylene-2,2′-bipyridylium dibromide) and paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) are very effective contact dessicants and are widely used in the preharvest dessication of various crops, for aquatic weed control, and for postemergent nonselective weed control. They are rapidly adsorbed by soils. An ion-exchange procedure for residue determination of diquat and paraquat in soil is available (Tucker *et al.*, 1967; Hance and McKone, 1970). Using a 25-g soil sample, a limit of detectability of about 0.1 ppm and recoveries of the order of 70–95% are possible (Hance and McKone, 1970). However, this method involves a time consuming ion exchange step and is likely subject to interferences from certain soil extractive in the colorimetric determination. Furthermore, Corwin *et al.* (1968) pointed out that certain reduced bipyridylium compounds may not follow Beer's law in aqueous solution.

Recently Soderquist and Crosby (1972) determined paraquat in water by a gas chromatographic technique using a flame ionization detector. Although the procedure has a limit of detectability in the order of 0.1 ppm, the recov-

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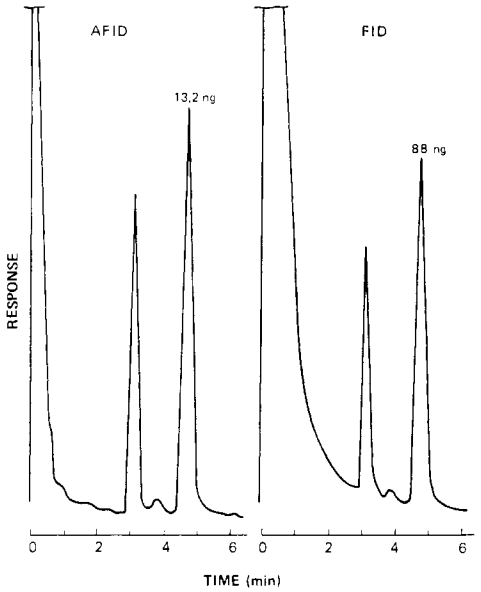

Figure 1. Gas chromatograms of hydrogenated diquat with the AFID and FID detectors. Gc conditions: glass column, 5 ft × 0.25 in. o.d. with 3% Carbowax 20M + 1% KOH on Chromosorb WHP; column temperature, 140°; detector and injector temperatures, 200 and 150°, respectively; carrier gas, nitrogen flow rate 50 ml/min; chart speed, 0.5 in./min; attenuation, FID 50 × 1 and AFID 20 × 1.

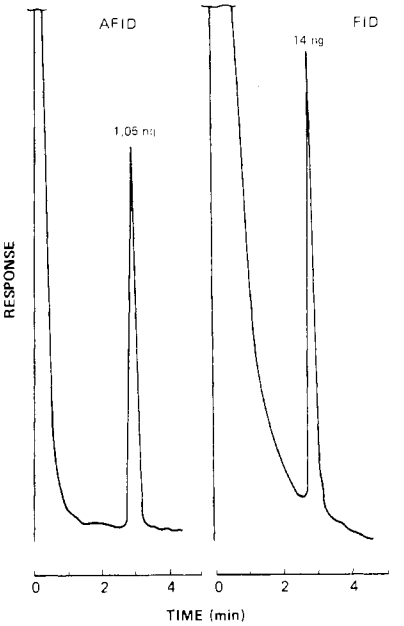

Figure 2. Gas chromatograms of hydrogenated paraquat with the AFID and FID detectors. Gc conditions same as in Figure 1.

eries from fortified water samples were poor (36–43%).

The purpose of this investigation was to develop a sensitive and reliable gas chromatographic procedure for the determination of diquat and paraquat residues in soils. The method described here involves catalytic hydrogenation of the acid extract of soil, extraction of the material into organic solvents, and analysis by gas chromatography.

EXPERIMENTAL SECTION

Chemicals. All solvents were pesticide grade and used as received. Analytically pure samples of diquat dibromide and paraquat dichloride were supplied by Chevron Chemical Co., Richmond Calif. Platinum oxide (Adam's catalyst) was purchased from Matheson Coleman and Bell Inc., Norwood, Ohio.

Hydrogenation of the Pure Herbicide. A simple apparatus somewhat similar to that described by Vogel (1966) was used for the catalytic hydrogenation. The herbicide (20 mg) in 30 ml of methanol was hydrogenated in the presence of 5 mg of platinum oxide (PtO_2). Hydrogenation was carried out at room temperature for 4 hr. The solution was concentrated to about 5 ml and 20 ml of 1 *N* NaOH solution was added (pH ~13). The mixture was extracted with hexane (10 ml × 3). Hexane was removed in a stream of dry air and the weight of the hydrogenated material was recorded. In another experiment the alkaline methanolic solution was first extracted with methylene chloride (10 ml × 3). Five milliliters of 1 *N* HCl was added to the methylene chloride extract and the solvent removed on a rotary evaporator at about 30°. The remaining aqueous portion was made alkaline (pH ~13) with 10 *N* NaOH and the mixture was then extracted with hexane (10 ml × 3). Hexane was removed as before and the weight of the dried material recorded.

Determination of Residues in Soil Fortified with Diquat and Paraquat. Soil samples were fortified at the 0.1- and 0.05-ppm levels using 20 g of soil. In all cases an aqueous solution of the herbicide was added to the soil and dried at room temperature. The soil was then mixed with 80 ml of 18 *N* H_2SO_4 in a boiling flask and heated under reflux for 5 hr. This step is essentially the same as used by other workers (Tucker *et al.*, 1967; Hance and McKone, 1970) for the extraction of diquat and paraquat from soils. The extract was filtered under suction through an acid-resistant filter paper and diluted to 500 ml with distilled water. A 125-ml aliquot (= 5 g of soil) was introduced into a hydrogenation flask containing 150 mg of PtO_2. Hydrogenation was carried out at room temperature for 1 hr. The pH of the hydrogenerated material was adjusted to about 13 with 10 *N* NaOH and the mixture transferred into a separatory funnel and extracted with three 70-ml portions of methylene chloride. The extracts were combined and 5 ml of 1 *N* HCl added and evaporated on a rotary evaporator at about 30°. The remaining aqueous portion was transferred into a 100-ml test tube, the flask was rinsed several times with a small volume of 0.01 *N* HCl, and the rinse collected in the test tube. Two milliliters of 10 *N* NaOH solution was added and the mixture was shaken with hexane (10 ml × 3). The phases were allowed to separate and the hexane layers were collected in a graduated centrifuge tube and concentrated with a stream of dry air and analyzed by gas chromatography.

Determination of Residues in Soils Collected from the Field. Surface samples were obtained from the experimental plots with a known history of the herbicide treatment. The samples were air-dried at room temperature, pulverized, screened through a 20-mesh screen, and mixed thoroughly. Subsamples (20 g) were used for the extraction of residues by the procedure described above. Acid extracts representing 1 g of soil were used for hydrogenation, using 25 mg of PtO_2 catalyst. Residues of diquat and paraquat were determined as described before.

Residues of diquat and paraquat in these soils were also determined by the ion-exchange method (Hance and McKone, 1970).

Effect of Time of Hydrogenation on the Recovery of the Herbicides. Increasing amounts of PtO_2 (5–40 mg) were added to 25-ml portions (= 1 g of soil) of the acid extracts of the field treated soil and hydrogenated for 3 hr. Residues of the herbicide were determined as described above.

Effect of Time of Hydrogenation on the Recovery of the Herbicides. Twenty-five milliliter portions (= 1 g of soil) of the acid extract were hydrogenated in the presence of 25 mg of PtO_2 for 0.25, 0.5, 1, 2, or 3 hr. At the end of

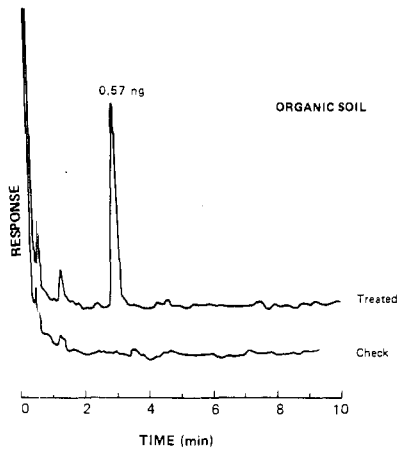

Figure 3. Gas chromatograms from determination of paraquat in organic soil (20 ml final volume, 1 μl injected). Gc conditions as in Figure 1.

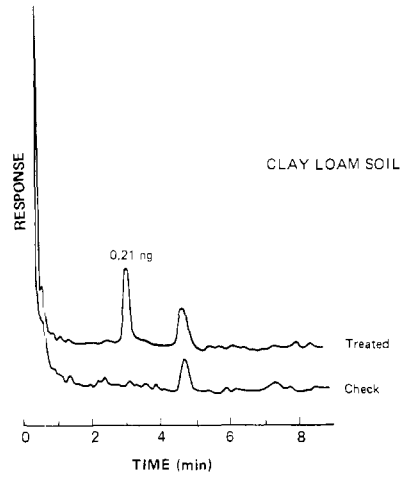

Figure 4. Gas chromatograms from determination of paraquat in clay loam soil (4 ml final volume, 1 μl injected). Gc conditions as in Figure 1.

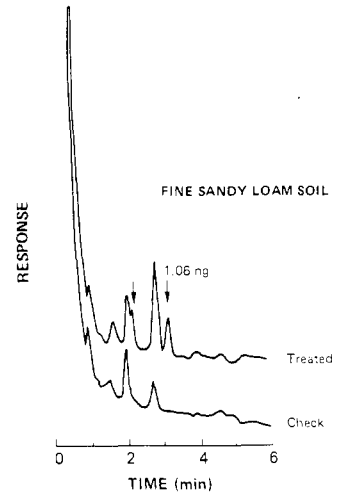

Figure 5. Gas chromatograms from determination of diquat in fine sandy loam soil (1.2 ml final volume, 4 μl injected). Gc conditions as in Figure 1 except column temperature 150° (retention time = 3.1 min).

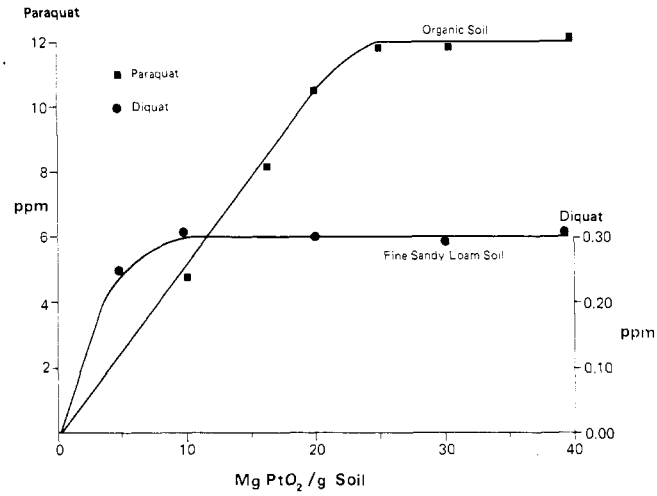

Figure 6. Effect of the amount of PtO_2 used in hydrogenation of soil extract on the recovery of herbicides. Hydrogenation was carried out for 3 hr.

the specified hydrogenation periods the herbicide residues were determined as described before.

Effect of pH of the Acid Extract on the Recovery of the Herbicide. The pH values of the 25-ml portions (= 1 g of soil) of the acid extract of soil prior to hydrogenation were adjusted to 1, 2, 5, 7, and 9 and the herbicide residues determined as described before.

Gas Chromatography. The gas chromatograph was a Pye Series 104, Model 124, fitted with a flame ionization detector (FID) and an alkali flame ionization detector (AFID) having a CsBr Annulus. Columns were all 5 ft × 0.25 in. o.d. glass tubes packed with 3% SE-30 (ultra phase), 5% Reoplex 400, 3% Carbowax 20M, or 3% Carbowax 20M + 1% KOH coated on 80–100 mesh Chromosorb WHP. The operating conditions were: column temperature, in the range 140–160° as indicated; detector and injector temperatures, 200 and 150°, respectively; carrier gas, nitrogen flow rate, 50 ml/min.

The concentrations of the herbicides in the soil extracts were determined by comparing the peak heights with those of the hydrogenated standards and correcting the value for the change in molecular weight on hydrogenation. The identity of the desired peak was proved by comparing its retention time and mass spectrum with those of the reference hydrogenated herbicides and by co-chromatography with the latter. For mass spectrum, an aliquot of the above solution containing approximately 1–2 μg of the hydrogenated material was injected into a gas chromatograph with a hydrogen flame detector coupled to a Du Pont Model 490 mass spectrometer.

All samples were analyzed in duplicate and average values are reported. Residues in soils are reported on an oven dry basis.

RESULTS AND DISCUSSIONS

Under the experimental conditions described diquat dibromide and paraquat dichloride yielded materials (hexane extraction) weighing 8.5 (75.4% yield) and 12.5 mg (82.0% yield), respectively. Preliminary experiments indicated that a hydrogenation longer than 4 hr did not result in increased yield of the hydrogenated materials. Extraction of the hydrogenated materials from the alkaline methanolic solution first with methylene chloride and then with hexane did not affect the yield. The products obtained from the catalytic hydrogenation of diquat and paraquat gave mass spectral parent peaks at m/e 194 and 196 and have been identified as perhydrodipyrido[1,2-*a*:2′,1′-*c*]pyrazine and 1,1′-dimethyl-4,4′-bipiperidine, respectively (Soderquist and Crosby, 1972). Gas chromatography of the former resulted in two symmetrical peaks (Figure 1) possibly the cis and trans isomers (Soderquist and

Table I. Gas Chromatographic Conditions, Calculated Least Detectable Amounts (lda),[a] and 50% Full-Scale Deflection (0.5 fsd) of Hydrogenated Herbicides with a Flame Ionization Detector (FID)[b] and an Alkali Flame Ionization Detector (AFID)[b]

			Hydrogenated diquat						Hydrogenated paraquat					
			FID			AFID			FID			AFID		
Column	Polarity	Column temp, °C	R_t,[c] min	0.5 fsd, ng	lda, 10^{-12} g/sec	R_t,[c] min	0.5 fsd, ng	lda, 10^{-12} g/sec	R_t,[c] min	0.5 fsd, ng	lda, 10^{-12} g/sec	R_t,[c] min	0.5 fsd, ng	lda, 10^{-12} g/sec
3% SE-30 (ultra phase)	Nonpolar	158	3.2	68	29.8	3.2	16	5.8	2.4	17	5.2	2.4	3.0	0.9
5% Reoplex 400	Intermediate	142	3.9	170	44.8	3.9	28	8.1	2.6	28	11.9	2.6	6.0	1.5
3% Carbowax 20M	Polar	161	4.0	88	24.4	4.0	10	2.3	2.6	16	3.8	2.6	3.0	0.8
3% Carbowax 20M + 1% KOH	Polar	140	4.8	81	20.9	4.8	10	2.3	3.0	9.8	3.3	3.0	0.9	0.3

[a] Calculated as 2× noise level. [b] Attenuation, FID 50 × 1; AFID 20 × 1. [c] Retention time.

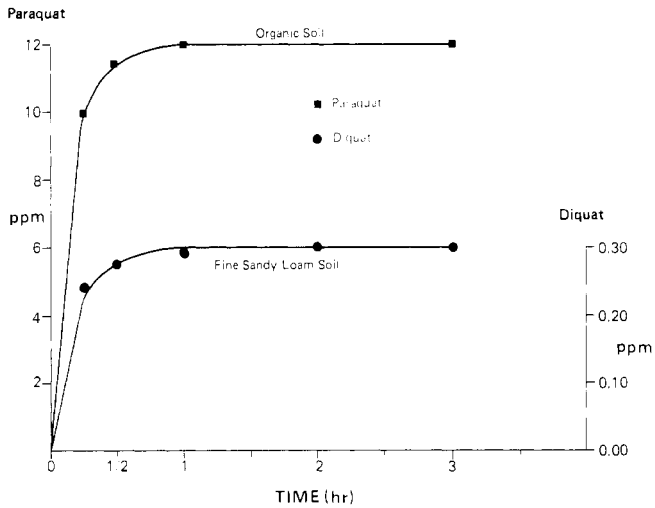

Figure 7. Effect of time of hydrogenation on the recovery of herbicides.

Crosby, 1972) while the latter produced a single symetrical peak (Figure 2). Since the ratio of heights of the two peaks (Figure 1) remained relatively constant for each column (SE-30, 1.0; Reoplex 400, 0.94; Carbowax 20M, 1.0; and Carbowax 20M + 1% KOH, 1.3) throughout the concentration range studied, quantitation of the data was obtained by the second peak (retention time = 4.8 min).

The comparative response characteristics of the two hydrogenated herbicides were determined on various columns using an AFID and a FID detector. The same column was connected to both the detectors through a splitter (1:1) and operated at its optimum temperature. Table I lists the retention time (R_t), calculated least detectable amount (lda), and the amount needed to give a response of 50% full scale deflection (0.5 fsd) for the hydrogenated compounds chromatographed under the condition described. The Carbowax 20M + 1% KOH column produced sharper peaks and was more sensitive and better in performance than the other columns examined in this study. Reoplex 400 and SE-30 columns exhibited slight tailing on the FID detector. Under the gc conditions described, the response of the hydrogenated diquat (concentration range used 1–105 ng) and hydrogenated paraquat (concentration range used 0.1–13 ng) on both detectors was linear. While either AFID or FID detector may be used in the determination, the former is preferred as it showed greater sensitivity. For the determination of residues in soils Carbowax 20M + 1% KOH column and AFID detector were chosen due to their better overall performance and greater sensitivity.

Table II. Recovery of Paraquat and Diquat from Fortified Soils

Soil	Herbicided added, ppm	% recovery
	Paraquat	
Clay loam	0.1	90.7
	0.05	91.9
Organic	0.1	94.6
	0.05	92.6
	Diquat	
Clay loam	0.1	89.6
	0.05	84.0
Organic	0.1	89.5
	0.05	86.7

The recoveries of the herbicides from the fortified soil samples at 0.1 and 0.05 ppm (based on 5 g of soil) ranged from 85 to 95% (Table II). It can be seen that under the experimental conditions described the procedure has a sensitivity of 0.05 ppm. However, concentration of the final solution five times may result in an improved sensitivity of about 0.01 ppm without any significant interference from soil background.

Figures 3, 4, and 5 show chromatographic tracings of the check and herbicides treated soil samples. A few unknown peaks appeared in the chromatograms but they did not interfere with the herbicide peak used for quantitation. The procedure is capable of detecting diquat and paraquat residues of about 0.4 and 0.03 ng in the injected sample, respectively. These values have been arrived at by taking the limit of detection in the injected sample as the level at which the signal is two times that of the background.

Table III lists the comparative residue values obtained by the method described in this paper and the ion-exchange procedure (Hance and McKone, 1970). The two methods appear to give similar results. It should be pointed out here that paraquat residue levels in the two soils used in this study were rather high (Table III). The ion-exchange procedure may be adequate for these levels of residues. However, the time involved for analysis by the proposed method was considerably less than by the ion-exchange procedure because of the lengthy cleanup step in the latter.

The effect of the addition of increasing amounts of the catalyst in the hydrogenation of soil extracts on the recovery of the herbicides residues is shown in Figure 6. There was an increase in the recovery of diquat and paraquat residues when the amount of the catalyst was increased from 2.5 to 10 mg and 25 mg/g of soil, respectively. Fur-

Table III. Comparative Residue Values for Paraquat and Diquat in Soils

Soil	Org matter, %	ppm: Gc method	ppm: Ion-exchange method
	Paraquat		
Clay loam	3.8	0.92	1.25
Organic	82.4	12.35	10.56
	Diquat		
Fine sandy loam	3.0	0.30	0.22

ther increase in the weight of the catalyst did not increase the recovery. The amount of the catalyst required for maximum recovery of residues depends on the soil type; organic soil required more catalyst, indicating an increasing demand for catalyst with an increase in organic matter contents of the soil. However, 25 mg of the catalyst per g of soil was adequate for the maximum recovery of residues from the three different types of soil. Under these conditions the hydrogenation of soil extracts was completed in 1 hr as further increase in time of hydrogenation did not improve the recovery (Figure 7). The maximum recovery of residues was not affected by varying the pH, over the range 1–9, of the acid extracts of soil prior to hydrogenation.

The method described in this paper is rapid and simple. No cleanup is necessary for routine soil samples. The lower limit of sensitivity for this method is approximately 0.01 ppm for diquat and paraquat.

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Estimation of Methionine and Cystine in Compounded Poultry Rations

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Reliable chemical methods for the estimation of sulfur-containing amino acids have been developed. With these procedures, the content of these two amino acids which are important for good egg production can be assessed in chick feeds. The determination of methionine was carried out by ascending thin-layer chromatographic separation. The separated spots were sprayed with ninhydrin and the color read by the Beckman DB-G spectrophotometer. The interfering spots were separated with the help of column chromatography over Dowex-1. Cystine was estimated according to the method of Schram *et al.* (Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57**, 33 (1954)). The method was based on the hydrolysis of oxidizing product, produced by performic acid treatment of the rations. The prepared hydrolysate was passed through a column of Dowex-2 and estimated according to the photometric ninhydrin method of S. Moore and W. H. Stein (*J. Biol. Chem.* **176**, 307 (1948)).

It is known that the nutritive value of a food protein depends on the amino acid content. Thus the evaluation of the nutritive quality of compounded poultry rations may be obtained by analyzing their amino acids.

The minimum requirements of methionine and cystine in a balanced diet for chickens were reported to be 0.45 and 0.35%, respectively (Bose, 1972). As these two sulfur-containing amino acids were among the more deficient nutrients in many poultry rations, the need for the estimation of these two substances was badly realized.

Some existing methods (Lavin, 1943; Toennis and Callan, 1939; Albanese *et al.*, 1944) were tried for the analysis of methionine in the compounded poultry rations and found unsuitable. The paper describes a simple method of methionine estimation by ascending thin-layer chromatography after necessary purification. Cystine was determined as cysteic acid using the column chromatographic method of Schram *et al.* (1954) and gave good results. This method was based on the performic acid oxidation of cystine followed by ion-exchange chromatography.

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ESTIMATION OF METHIONINE

Reagents and Apparatus. Thin-layer plates consisted of thin layers of 0.4 mm thickness on 20 × 10 cm frosted glass plates using silica gel (Gouri Chemical, 28/3D Haray Kristo Sett Lane, Calcutta 50, India), and dried overnight at room temperature. The solvent system consisted of a mixture of *n*-butyl alcohol, glacial acetic acid, and water (1:4:1, v/v). The developing reagent was a 0.1% solution of ninhydrin in *n*-butyl alcohol. Solutions of L-methionine hydrochloride in the range of 0.030–0.185 mg/ml in water were used as standards.

Methods. *Preparation of the Sample.* Finely ground fat-free material was hydrolyzed with 6 *N* HCl for 18–24 hr at 110°. The contents were then cooled to room temperature