Improved Total Phosphorus Method for Determination of Disyston in Dried Sugarbeet Pulp

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Measurement of total phosphorus by phosphomolybdate-blue in isobutanolbenzene medium for the determination of residues of Disyston, 0,0-Diethyl S-[2-(ethylthio) ethyl] phosphordithioate and its oxygen analogs in plant material has been described by Anderson (1)[2]. The method was found to be inadequate when applied to dried sugarbeet pulp. The inadequacies were in the procedures of extraction and total phosphorus determination. This paper presents a method which has simplified the original procedure and yet improved its precision and accuracy for dried sugarbeet pulp.

Method

Apparatus and Materials

(a) Osterizer blender for grinding sample. - Model No. 10, 115 volts, 2.2 amps AC-DC, mounted with one quart, standard, pyrex container, manufactured by John Oster Mfg. Company, Milwaukee, Wis., or equivalent.

(b) Waring Laboratory Blendor for extracting sample. - Consists of three parts: (1) Explosion-proof motor, GE, Model No. 5BA60VL22, 1/5 HP, 115 volts, 4.2 amps and 60 cycles DC: (2) Stainless steel container, type 302, one quart; (3) Explosion-proof switch, obtainable from Waring Products Company, Winsted, Conn., or equivalent.

(c) Chromatographic columns. - K-42028, with 2 A Teflon plug, size D-2. Kontes Glass Company, Vineland, New Jersey.

(d) Kjeldahl digesting unit. - S-63200, Micro, Pregl, 6 place, gas heated, E. H. Sargent and Company.

(e) Spectrophotometer. - Model DU, Beckman Instruments, Inc.

(f) High vacuum evaporator. - VE-1000-B, Rinco Instrument Company, Inc., Greenville, Ill.

(g) Digestion tubes. - Folin-Wu, 50 ml.

(h) Separatory funnels. - Kimax 29048-F, 30 ml, with Teflon plug, Kibzle Products, Toledo, Ohio. (For use, mark at the 15 ml level.)

(i) Activated carbon. - Darco 20 $\times$ 40, granular, Atlas Chemical Industries Inc., Wilmington, Del.

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[2] Numbers in parentheses refer to literature cited.

(j) Aluminum oxide. - Merck 71695, acid washed, chromatographic grade, A. Daigger and Company, Chicago, Ill.

(k) Superbrite glass beads. - Size 130-5005, Serial No. P-4495, Minnesota Mining and Manufacturing Company, St. Paul, Minn.

Reagents

(a) Acetone, chloroform. - Reagent grade and redistilled, discard the very first and very last parts of the distillate, use only mid-part of distillate.

(b) Iso-butyl acetate. - Reagent grade.

(c) Molybdate solution. - Dissolve 10.69 g of analytical reagent grade ammonium molybdate tetrahydrate, $(NH_4)_6\ Mo_7O_{24}\cdot 4H_2O$, in double distilled water and dilute to 1 liter.

(d) Nitric acid. - Reagent grade, 8 N.

(e) Perchloric acid (70%).

(f) Hydrochloric acid. - Reagent grade. Concentrated and 2 N.

(g) Sulfuric acid. - Reagent grade, 1 N.

(h) Sodium hydroxide. - Reagent grade, concentrated and 0.1 N.

(i) Thymol blue (0.1%). - Dissolved in ethanol.

(j) Phosphorus stock solution. - Dissolve 0.4393 g of reagent grade, oven dried, KH_2PO_4 in double distilled water and dilute to 1 liter. This stock solution is 100 ppm in phosphorus. Working solution: dilute 2, 4, 6, 8, and 10 ml of stock solution to 100 ml, respectively. They are 2, 4, 6, 8, and 10 ppm phosphorus.

(k) Stannous chloride. - Dissolve 10 g of stannous chloride dihydrate in 25 ml of HCl in a brown bottle. For use: dilute 1 ml to 20 ml with 1 N H_2SO_4.

Extraction

Grind 100 g dried beet pulp or pellets in an Osterizer blender for 5 minutes. Blend only 25 g of the ground sample with 100 ml double distilled water and 200 ml acetone in a Waring Laboratory Blendor for 5 minutes. Filter through Whatman No. 5 filter paper on a Buchner funnel. Record the recovered volume of filtrate. Shake the filtrate with 250 ml chloroform. Repeat the extraction with two 50 ml portions of chloroform. Combine all chloroform extracts and evaporate to dryness in a high vacuum evaporator at temperature of 40° C.

Chromatographic Procedure

Chromatographic column packing procedure is according to Anderson (1). Place a plug of glass wool in the bottom of column. Open stopcock slightly. Pour 15 g Superbrite beads into column. Wash column with 50 ml acetone. Drain off some acetone. Close the stopcock. Add 10 g aluminum oxide. Wash down with an-

other 50 ml acetone. Finally add 10 g Darco carbon into column. Tap the sides of column to make each layer as tight as possible. Rinse the packed column with 200 ml acetone at a flow rate of 3-4 ml per minute. During the column rinsing tap the sides several times.

Before the acetone rinse penetrates the carbon layer by not more than one-half inch rapidly transfer the vacuum dried sample from the evaporatory flask to the column by means of 10 ml of chloroform-acetone (30:70, v./v.). Rinse the evaporatory flask with 3 portions of 5 ml acetone and successively pour to column before sample starts to enter into column. Collect the eluate. Pour 300 ml acetone to the column. Evaporate the eluate to 1-2 ml in a 250 ml evaporatory flask under vacuum.

Digestion

Transfer the concentrated sample to a digestion tube. Use two portions of 2 ml acetone to remove the remaining sample. Evaporate it down to about 0.5 ml in a water bath (40° C) with a jet of air. Cool the content to room temperature. Add in order, 2 ml of 8 N HNO_3, chill in ice water, 0.5 ml of 70% perchloric acid, and a glass bead. Heat gently on a Kjeldahl digestion unit to boiling. Shut off the flame. Allow the billowy brown fume to subside. Continue to digest the sample until no more white fume remains. Time required for digestion is about an hour. Final volume of the digest is about 0.5 ml.

Colorimetric Determination of Phosphorus

Allow the digest to cool. Transfer it with 3 portions of 2 ml of double distilled water to a 30-ml separatory funnel containing 6 ml of molybdate solution. To the mixture add 2 drops of thymol blue, and swirl the mixture well. Neutralize the mixture first with concentrated NaOH to turn indicator from red to yellow. Continue neutralizing with 0.1 N NaOH until the yellow color remains after shaking (2). To the neutralized mixture add 1 ml concentrated HCl. Bring volume to 15 ml mark with double distilled water. Shake the mixture with exactly 10 ml iso-butyl acetate for 1 minute to extract phosphomolybdic acid from aqueous phase to organic phase (3). Discard the aqueous phase. Wash the organic phase with 5 ml of 2 N HCl for 30 seconds. Again discard the aqueous phase. Remove any remaining droplets of water from stem of funnel. Drain the organic phase to a 25 ml graduate cylinder. Its volume should be still 10 ml. Add 6 drops of dilute stannous chloride solution. Read absorbance against a water blank at 730 mμ on a Beckman DU spectrophotometer. Reading is best taken after blue color has developed for 10 minutes but not over 25 minutes.

Preparation of Standard Curve

Pipette 1 ml of each working standard phosphorus solution to five 30 ml separatory funnels containing 6 ml molybdate solution, respectively. To the solution add 7 ml double distilled water and 1 ml concentrated HCl. Allow solution to stand for 5 minutes. Shake the solution for 1 minute with exactly 10 ml iso-butyl acetate. Then follow through rest of steps as previously described.

Calculation

$$\text{ppm Disyston} = \frac{(A)}{(B)} \frac{(274)}{(31)} \frac{(4)}{(C)}$$

A = Absorbance of sample.

B = Absorbance of a 4-microgram phosphorus standard.

$$C = 25 \text{ g} \times \frac{\text{ml of extract recovered}}{\text{300 ml of extraction solvent}}$$

Molecular weight of Disyston = 274.

Results and Discussion

The apparent residues of Disyston in controlled dried beet pulp varied with sample size and sample form (molasses pulp or plain pulp). In dried molasses pulp 50 g samples had apparent residues from 0.1 to 0.4 ppm, 25 g samples had 0.0 to 0.2 ppm; whereas dried plain pulp samples were from 0.5 to 1.0 ppm for 50 g samples and 0.2 to 0.5 ppm for 25 g samples. It appeared that a sample weight of 25 g assured the method a better sensitivity. This was further evidenced when percent recovery of added Disyston from 25 g dried molasses sample versus those of 50 g and 12.5 g were compared (Table 1).

Extraction solvent used here was 100 ml water and 200 ml acetone to a sample of 25 g. This aqueous acetone proved itself a most promising extractant over Anderson's solvents (1) as shown in Table 2.

Acids used for digestion of organic phosphate pesticide in this paper were similar to those of Stellar and Curry (4). The digest was brought to neutral with NaOH (2). Again the digest was acidified by 1 ml of concentrated HCl to 0.8 N. The purpose

Table 1.—Percent recovery of added Disyston from dried molasses sugarbeet pulp.

Sample grams	Disyston ppm, added	% Recovery
50	1.0	147
	0.5	140
25	1.0	100
	0.5	116
12.5	1.0	98
	0.5	50

Table 2.—Comparison of extraction solvents

Extraction by	Sample grams	Disyston ppm, added	% Recovery[1]
Anderson's Moist[2]	25	.5	96
Anderson's dry[3]	25	.5	87
Modified moist[4]	25	.5	100

[1] Average of 2 runs.
[2] 140 ml water and 250 ml acetone, blended for 5 minutes.
[3] 400 ml chloroform reflux extraction for 24 hours.
[4] 100 ml water and 200 ml acetone, blended for 5 minutes.

of doing this is to insure the same acidity for each sample as well as for standards.

The phosphomolybdic acid was reduced to blue color in a medium of isobutyl acetate (3) instead of isobutanol-benzene (1). Because the selectivity of iso-butyl acetate for extraction of phosphomolybdic acid is as good as isobutanolbenzene, yet with no

Table 3.—Percent recovery of added oxygen analogs of Disyston from dried sugarbeet pulp.

Sample 25 g	Sulfone		Sulfoxide	
	ppm, added	% recovery	ppm, added	% recovery
Molasses pulp	0.2	120	0.2	100
	0.5	100	0.5	112
	1.0	102	1.0	100
Plain pulp	0.2	120	0.2	124
	0.5	89	0.5	114
	1.0	104	1.0	110

loss of the phosphate to the aqueous phase for iso-butylacetate is insoluble in water. Also phosphorus determined in iso-butyl acetate should be free of interference from most ions (3).

The percent recovery of oxygen analogs of Disyston obtained with the improved method showed excellent results (Table 3). Those had recovery slightly over 100%, possibly resulting from glassware or unusual samples of high apparent residues.

Literature Cited

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