Analysis of Difenzoquat Herbicide in Wheat Products by Reversed-Phase Liquid Chromatography

Difenzoquat (1,2-dimethyl-3,5-diphenylpyrazolium methyl sulfate), a herbicide used for the control of wild oats, was determined in wheat products by reversed-phase ion-pair chromatography employing a LiChrosorb RP-8 column and a mobile phase of 70% acetonitrile in water containing 0.01 M sodium octanesulfonate, adjusted to pH 3.0. Samples of bread, cereal, and flour were extracted with acidified acetonitrile, which was cleaned up by aqueous/organic partitions at acidic and basic pH values. The organic extract was then further purified by passing through an alumina column. The elution fraction containing the difenzoquat was evaporated to dryness and dissolved in the mobile phase for analysis. Recoveries of spiked samples over the range 0.5–10.0 ppm averaged 88.6% with a range of 83.5–99.2%. At 0.1 ppm, the average recovery was 75.3% with a range of 68.1–85.9%. The detection limit was estimated to be ~0.02 ppm in the samples studied.

Difenzoquat (1,2-dimethyl-3,5-diphenylpyrazolium methyl sulfate) is a quaternary ammonium compound used as a herbicide for control of wild oats (*Avena fatua*, *Avena sterilis*, and *Avena ludoviciana*) in cereal crops, especially wheat in the prairie regions of Canada. Its herbicidal properties have been described by O'Hare and Wingfield (1973) and Shafer (1974). The acute oral LD_{50} for the technical-grade material is 470 mg/kg of body weight in male albino rats. Difenzoquat is one of five wild oat herbicides which have become of interest due to their heavy usage in the prairie provinces. No methodology has yet been reported for residues of difenzoquat in wheat products. This report describes a liquid chromatographic (LC) method for the direct analysis of the herbicide in three types of wheat products, including flour, bread, and cereal. It makes use of the ion-pairing behavior of the pyrazolium ion of difenzoquat with octanesulfonate. A recent publication has appeared on the analysis of difenzoquat in formulations employing LC and using ion pairing with phosphate (Barry and Pike, 1980). It is possible that this system would also be satisfactory for residue analysis.

It is intended that the LC method described herein be integrated to some degree with LC methodologies, including sample extractions and cleanup where possible for the other four wild oat herbicides. These include barban (Lawrence et al., 1980a), bromoxynil octanoate, benzoylprop ethyl (Lawrence et al., 1980b), and asulam (Lawrence et al., 1980c). A selective screening method by thin-layer chromatography has recently been reported for photosynthesis-inhibiting herbicides such as ureas, triazines, uracils, and anilides which can detect these compounds in the picogram range (Lawrence, 1980). However, this technique was found unsuitable for the wild oat herbicides due to their different mode of herbicidal action.

EXPERIMENTAL SECTION

Reagents. Distilled-in-glass grade solvents were used for all sample extractions and preparation of the standard solutions. A stock solution of difenzoquat was prepared in distilled, deionized water at a concentration of 1.0 mg/mL. Spiking solutions were prepared from this by dilution with acetonitrile. Standards for LC were prepared by dilution of the stock with mobile phase.

The wheat products analyzed were whole wheat cereal, flour, and bread, as well as samples of refined white flour and bread, all locally purchased at retail outlets. Spiked samples were prepared by adding ~1 mL of spiking solution to 10 g of sample, mixing well, and permitting the mixture to sit for at least 2 h before extraction.

Liquid Chromatography. The LC system consisted of an Altex Model 110A pump for solvent delivery and a Waters Model 450 variable-wavelength detector (8-μL cell volume) set at 265 nm and 0.04 or 0.08 absorbance units (AU) full scale. A 1-mV recorder was used instead of one of 10 mV which resulted in a scale expansion of ×10, yielding an actual recorder full-scale range of 0.004–0.008 AU. Chromatography was carried out at ambient temperature on a 25 cm × 4.6 mm (i.d.) LiChrosorb RP-8 column with a mobile phase consisting of acetonitrile–0.01 M sodium octanesulfonate (7:3 v/v) and adjusted to pH 30 with 0.1 M H_3PO_4. The flow rate was set to 1.0 mL/min. The mobile phase was filtered through a 0.45-μm Millipore type HA filter before use. For routine operation ~50 mL of the mobile phase was first pumped through the column and discarded. After this the mobile phase was recycled. Normally, 1 L of the mobile phase was prepared at a time and was useful for at least 1 week when actual samples were injected. Overnight the flow rate was reduced to 0.2 mL/min but never shut completely off. During the weekend, or when the apparatus was not in use on a daily basis, the system was thoroughly rinsed with water, followed by ~100 mL of acetonitrile. With this procedure, the column maintained its integrity for at least 6 months of daily operation. Injection volumes were normally 50–100 μL via a Rheodyne syringe-loop injection port (100-μL loop). Difenzoquat eluted with a retention time of ~12 min.

Sample Extraction. Ten grams of wheat was placed in a 250-mL beaker, followed by the addition of 20 mL of 2.0 N HCl. The mixture was stirred with a short glass rod which was left in the beaker. The contents were then sonified in an ultrasonic bath for 10 min. The mixture was stirred again, permitted to stand for an additional 30 min, and then sonified for an additional 5 min. After this treatment, 100 mL of acetonitrile was added to the beaker and the mixture transferred to a 600-mL stainless steel cup of a Sorvall Omnimixer homogenizer. The mixture was homogenized for 1 min at medium speed (setting 5), and then it was suction filtered through a 150-mL medium-porosity sintered glass funnel into a 250-mL flask. The

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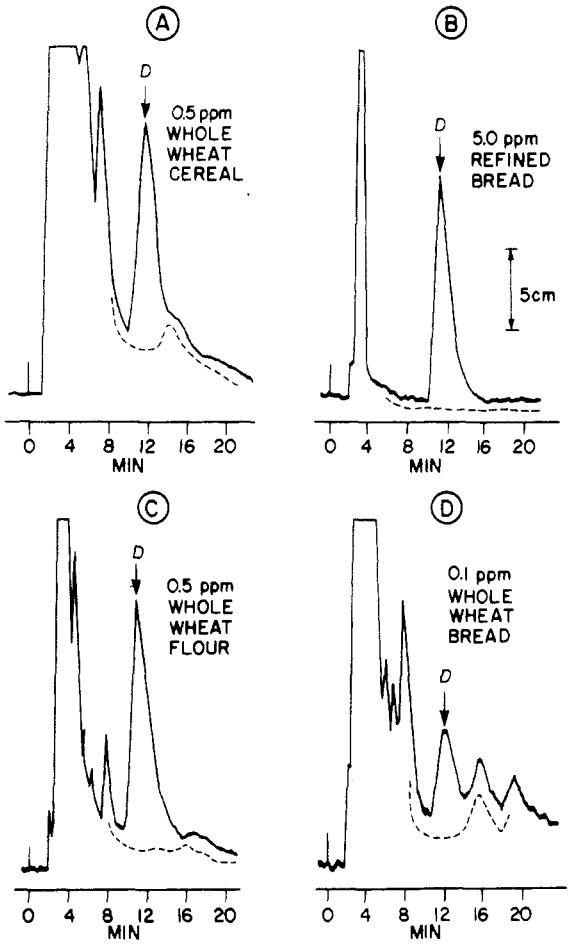

Figure 1. Chromatograms of wheat products spiked at levels of 0.1–5.0 ppm with difenzoquat. Chromatography conditions were as described in the text. (A) 400 mg of equivalent sample injected. (B) 40 mg of equivalent sample injected. (C) 400 mg of equivalent sample injected. (D) 500 mg of equivalent sample injected. (A), (B), and (C) were monitored at 0.008 AU recorder full scale. (D) was monitored at 0.004 AU recorder full-scale. Dashed lines represent the response from the sample blanks.

volume of the filtrate was adjusted to exactly 150 mL with acetonitrile.

Partition. An aliquot representing 4.0 g of sample was placed in a 100-mL round-bottom flask and evaporated with a rotary vacuum evaporator at 45 °C to an aqueous residue. Four milliliters of distilled water was added and the pH adjusted to 8–9 by using 2.0 N NaOH (or 0.2 N NaOH near the end point). The solution was quantitatively transferred to a 125-mL separatory funnel containing 10 mL of hexane. The contents were shaken well by hand for 1 min and the hexane layer was discarded. The aqueous layer was subsequently partitioned with 3 × 40 mL of methylene chloride. The combined methylene chloride extracts were passed through ~2 g of anhydrous sodium sulfate (contained in a small filter funnel) and then evaporated to ~25 mL.

Column Chromatography. The 25 mL of methylene chloride extract was added to the top of an alumina (neutral; 5 g) column and permitted to flow into the column bed. This was followed by 40 mL of 1% methanol in methylene chloride which was discarded. The difenzoquat was removed from the column with 40 mL of 6% methanol in methylene chloride which was collected in a 100-mL round-bottom flask and evaporated to dryness under vacuum. Traces of solvent vapors were removed from the flask with a gentle stream of nitrogen. The residue was made up in an appropriate volume (usually 1–20 mL for herbicide concentrations of 0.2–10.0 ppm) of the mobile phase for LC analysis (duplicate injections).

Table I. Percent Recoveries of Difenzoquat in Wheat Products

	% recovery at a spiking level, ppm, of				
sample	0.1	0.5	1.0	5.0	10.0
whole wheat cereal	68.1	87.7	83.5	99.2	91.7
refined white flour	79.7	92.5, 89.9	85.3, 81.8	87.7, 83.0	86.3, 86.2
refined white bread	85.9	87.2	91.2	87.2	84.8
whole wheat flour	74.6	97.6, 90.4	91.3, 91.5	89.6, 87.4	80.4, 85.4
whole wheat bread	68.1	90.9, 93.4	86.4, 88.7	89.9, 89.9	86.5, 87.1

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of wheat products spiked at various levels with difenzoquat. The minimum level quantitatively studied was 0.1 ppm while 0.02 ppm was the estimated detection limit. The wavelength, 265 nm, was selected for monitoring the LC effluent even though 255 nm is the absorbance maximum of difenzoquat. The reason for this choice was that sample coextractives absorbed significantly less at 265 nm, resulting in cleaner chromatograms, especially at the lower spiking levels. At the same time, the response of the detector to difenzoquat dropped by ~10%; however, this was more than compensated for by the reduction in absorbance due to coextractives.

It was found that traces of residual organic solvent in the final sample solution seriously affected the chromatography by distorting the difenzoquat peak. Thus, all traces including vapors were removed from the residue after the alumina column cleanup by flushing the container with nitrogen gas. It has been shown that small quantities of moderately polar solvents such as chloroform effect chromatographic separations in reversed-phase LC (Ryan and Pilon, 1980).

Table I shows recoveries obtained for five wheat products spiked at 0.1–10.0 ppm. At 0.5 ppm or above, recoveries were 80% or greater. However at 0.1 ppm recoveries were somewhat lower but still greater than 68%.

Initially, the extraction solvent employed was 20% methanol in methylene chloride which was recommended by American Cyanamid Co., the producer of difenzoquat, for extraction of the herbicide from grains. While this worked for samples which were extracted immediately after spiking, it was not useful if several hours passed before extraction. Recoveries (at 1.0 ppm) were found to drop to ~50–60% after permitting the spiked samples to sit at room temperature for 5 h. If the spiked samples were left overnight, recoveries dropped to <40%. As a result, the methanol–methylene chloride extraction procedure was not considered satisfactory. The 2 N HCl treatment of the flour and bread samples was found effective in extracting difenzoquat from the same samples. Also, 1 N HCl was less effective as was 2% HCl in acetonitrile. Recoveries from samples spiked 5 days before being extracted were well over 80% when the 2 N HCl treatment was included. The minimum detectable amount of difenzoquat standard was ~6 ng (2:1 signal:noise ratio) under the detector and chromatographic conditions used. No difenzoquat was found to occur above the detection limit in any of the retail samples analyzed.

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Received for review November 10, 1980. Accepted March 18, 1981.

Lead Contents of Commercially Canned Single-Strength Orange Juice Stored at Various Temperatures

The lead contents of commercially canned single-strength orange juice varied within a narrow region (0.02–0.32 mg/kg of juice). Analysis of 168 samples showed that only four samples exceeded by 0.01–0.02 ppm the stringent tolerance level of 0.3 ppm established by FAO/WHO for canned baby juices. No statistical relationship was observed between storage temperature and lead content with commercial samples. Our data suggest that the variability in the lead contents of commercially canned orange juice was primarily due to the amount of solder (splashings and seepage through the side seam) exposed to the juice.

Lead is nutritionally a nonessential mineral that shows moderate toxicity. The toxic nature of lead is due to its binding to active sites of important enzyme systems in cells and to some ligands in the cell membrane (Stoewsand, 1980). Lead in food essentially originates from three sources, namely, (1) the natural lead content of food, (2) environmental pollution (lead dust from automobile exhausts or lead in runoff water enters food crops in certain areas), and (3) food processing activities involving the use of lead (Department of Health and Human Services, 1979). The most important source of added lead in a canned food product is not from the container (cans are constructed from plain carbon steel plates with a thin coating of tin) but from the solder (98% lead; 2% tin; American Can Company, 1973) used to seal the side seam. The Department of Health and Human Serivces (1979) estimates that ~14% of the total lead ingested by humans comes from the solder of canned foods. In this communication we report the results of temperature effects on the lead contents of commercially canned single-strength orange juice (SSOJ) that was stored for 12 weeks.

MATERIALS AND METHODS

Sample and Storage Treatment. Commercially cannned SSOJ was obtained from four processors located in Florida. SSOJ in 46-oz cans was taken directly from production lines and stored for 12 weeks at 21, 27, 32, 38, 43, and 49 °C.

Methods. Fifty grams of SSOJ was removed from a 46-oz can and placed in a tared platinum crucible. Charring of the sample, followed by ashing at 510–525 °C, was accomplished by a procedure previously described (Nagy et al., 1980). After ashing, the sample was dissolved in 2 mL of 1:1 concentrated HNO_3 and made up to a 100-mL volume with distilled water, and a portion transferred to a 60-mL polyethylene bottle. Replicate samples were run at each storage temperature. Nitric acid was distilled from glass at atmospheric pressure. Lead was determined by flameless atomic absorption spectroscopy by the method of Rouseff and Ting (1980).

RESULTS AND DISCUSSION

The concentration of lead in canned single-strength orange juice is due to (a) the natural lead content of orange juice and (b) lead derived from the solder. Because of the diversity of samples, we did not determine the natural lead levels for these juices prior to commercial canning. There is limited information on the natural lead content of Florida orange juice; Roberts and Gaddum (1937) reported 0.03–0.14 ppm of lead in blood orange juice, whereas McHard et al. (1980) reported values less than 0.1 ppm of lead (based on single strength) for Florida concentrated orange juices that were reconstituted. Recently, Rouseff and Ting (1980) reported an average value of 0.06 ppm of lead for noncanned single-strength grapefruit juice.

Storage of juices for a 12-week period (Table I) at temperatures ranging from 21 to 49 °C showed no statistical relationship (analysis of variance, 1% level of significance) between storage temperature and lead content. This lack of correlation was apparently due to the nonuniformity of the cans. In a study on canned fruit, Thomas et al. (1975) found the intercan variations of lead (solder) to have a coefficient of variation range from 18.2 to 97.8%. Rouseff and Ting (1980) found during a carefully controlled temperature experiment with canned grapefruit juice that considerable variation existed in the lead contents. This variation was primarily due to differences in solder splashings and solder seepage through the side seam. On the basis of these observations, Rouseff and Ting con-

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