# Determination of 2,4-Dichlorophenoxyacetic Acid in Wheat Grain

A method for determining 2,4-dichlorophenoxyacetic acid (2,4-D) in wheat grain, with a sensitivity of 0.05 ppm, involves continuous liquid–liquid extraction of the acidified wheat grain with diethyl ether, followed by esterification of the 2,4-D with $BCl_3$ in 2-chloroethanol and gas chromatography of the ester. Interfering substances are removed by a Florisil column cleanup. Average recoveries of 89% are obtained from wheat grain to which known amounts of 2,4-D have been added.

Established methods (Yip, 1962, 1971; Marquardt et al., 1964) for extraction of 2,4-dichlorophenoxyacetic acid (2,4-D) from plant materials involve acidification of an aqueous homogenate of the sample, followed by extraction of the 2,4-D with an organic solvent. It has been our experience that for oily crops such as wheat grain, direct extraction methods such as these sometimes afford emulsions that are troublesome to handle and difficult to break. Satisfactory quantitative removal of 2,4-D from wheat grain has been obtained using a continuous extraction apparatus first described by Kahn and Wayman (1964). The extracted solution overflows through a side arm of the extraction flask to a distillation flask, and the solvent is recycled to the extraction flask by distillation in a closed system. The powdered sample, in aqueous suspension, is acidified and extracted with diethyl ether. The 2,4-D is determined by gas chromatographic analysis of its 2-chloroethanol ester, as described by Woodham et al. (1971). Interfering chromatographic peaks, probably due to naturally occurring acid components of the grain, have been observed; this interference can be removed by Florisil column chromatography of the esterified solution.

## EXPERIMENTAL SECTION

**Extraction.** The liquid–liquid extraction apparatus is shown in Figure 1. Approximately 40 g of wheat grain was ground to a fine powder in a CRC Micro-Mill. Exactly 25 g of the powder was weighed directly into 2000-ml erlenmeyer flask (A). A Teflon-coated stirring bar and 1600 ml of distilled water were added to the flask, and the contents were stirred until the sample was well dispersed. The stirred mixture was acidified by careful addition of 4 ml of concentrated HCl, and stirring was continued for 15 min.

A hollow glass tube (B, Figure 1), which is flared at one end and perforated with holes at the closed end, was carefully suspended into the vertical portion of adaptor C so that the flared end will rest as shown. The setup (B and C) was placed onto flask A and fastened with holding springs. A 250-ml erlenmeyer flask (D) containing boiling beads was attached and fastened with springs to the side arm in C. Nanograde diethyl ether was poured through the top of C and was allowed to drain through B by bubbling out into the aqueous layer. Ether was added until about 200 ml from the upper layer in A had overflowed through the side arm into D. A water-cooled condenser (E) was placed on C. The ether in D was heated, under reflux, by a warm water bath. The return flow of ether to distillation flask D was regulated at a rate of approximately 5 drops/sec. After the stirred aqueous suspension had been extracted for 3 hr, condenser E was removed from the system, and the boiling ether in distillation flask D was allowed to evaporate to a volume of approximately 50 ml.

This extract was then filtered through a layer of anhydrous sodium sulfate, and the dried extract was collected in a 125-ml erlenmeyer flask. The flask and the funnel containing the sodium sulfate were washed with two 25-ml portions of nanograde diethyl ether. A Snyder column was placed on the flask containing the extract and the washings. The ether solution was concentrated on a steam bath to a volume of approximately 5 ml, and this was transferred quantitatively to a 15-ml graduated centrifuge tube. The resulting solution was evaporated to dryness, and the residue esterified and washed according to the method of Woodham et al. (1971).

**Florisil Column Cleanup.** The column was prepared by placing a small wad of hexane-washed glass wool into the bottom of a 5.75 in. disposable capillary pipet. Florisil (Floridin Co., magnesium silicate) was added with gentle tapping of the column until a height of 2 in. was reached. Anhydrous granular $Na_2SO_4$ was then added to an additional 0.5 in. The column was prewashed with 10 ml of nanograde diethyl ether followed by 10 ml of nanograde hexane, care being taken that the column was not allowed to dry.

One milliliter of the hexane extract of the esterified mixture was placed onto the top of the column. After the sample drained through the $Na_2SO_4$ layer, the column was first eluted with hexane until a volume of 5 ml was collected. The column was then eluted with a mixed solvent consisting of hexane–diethyl ether in a ratio of 24:1 (v/v) until 5 ml of eluate was collected. This portion of eluate was evaporated to 1 ml and was ready for gas chromatographic analysis.

**Gas Chromatography.** An injection volume of 5.0 $\mu$l was used to obtain the gas chromatographic analysis of the individual wheat samples. The amount of 2,4-D in the residues was determined by direct comparison of each sample with the recovery value obtained for a known amount of 2,4-D standard. An electron-capture detector was used. The operating parameters for the gas chromatograph are

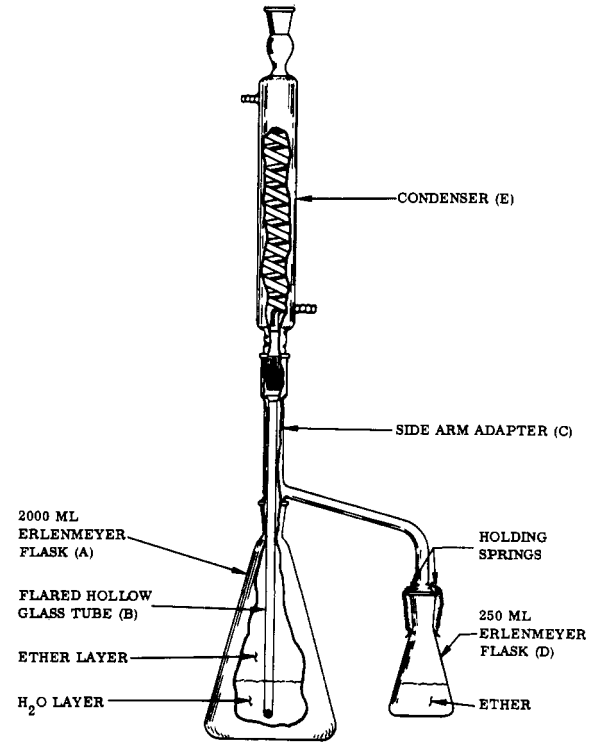


**Figure 1.** Liquid–liquid extraction apparatus.

**Table I**

| | 1.5% OV-17–1.95% QF-1 | 3% OV-1 |
|---|---|---|
| Instrument | Micro-Tek | Micro-Tek |
| Column | 6 ft, glass | 6 ft, glass |
| Support | Supelcoport (100–120 mesh) | Chromosorb W (100–120 mesh) |
| Carrier gas | 5% methane–argon | 5% methane–argon |
| Flow rate | 50 ml/min | 75 ml/min |
| Tank pressure | 50 psi | 50 psi |
| Temperature column | 160° | 160° |
| Detector | 200° | 200° |
| Injection port | 240° | 240° |
| Chart speed | 15 in./hr | 15 in./hr |
| Sensitivity | $(1.8 \times 10^{-8}) \times 32$ | $(1.8 \times 10^{-8})^2 \times 16$ |

**Table II. Recovery of 2,4-D from Wheat Grain**

| Amt fortified, ppm | % recovery | Amt fortified, ppm | % recovery |
|---|---|---|---|
| 0.05 | 88.0 | 0.5 | 92.1 |
| 0.1 | 94.7 | 1.0 | 89.8 |

shown in Table I. For the parameters listed, the retention times of the 2,4-D standard on the mixed and OV-1 columns are 15 and 22 min, respectively.

RESULTS AND DISCUSSION

Recovery experiments were performed by adding 0.05 to 1.0 ppm of standard 2,4-D to wheat samples before extraction was begun. The efficiency of the overall procedure was determined by comparing the peak heights of the standards with those from fortified samples. Unfortified samples were processed in an identical manner and were used as controls. The recoveries were determined by comparing the differences in peak heights between the fortified sample and the control with the standard.

The entire process, including the column cleanup which removes interfering chromatographic peaks, yielded recoveries between 77 and 95%. The results of this recovery study are summarized in Table II, and the values for repetitive runs are listed in Table III. The lower limit of detection for the procedure is about 0.05 ppm of 2,4-D. Some batches of ether, when run through the entire procedure as a solvent check, show interfering peaks on the chromatograms. A purification step, consisting of passing ether through a column of Woelm basic alumina (W-200, activity I, prewashed with ether), removes the interfering substances. Approximately 50 g of alumina will purify about 1000 ml of ether.

**Table III. Recovery of 2,4-D from Wheat Grain, Repetitive Runs**

| Sample no. | Amt 2,4-D fortified, ppm | % Recovery | % Av |
|---|---|---|---|
| 6071 | 1.0 | 86.5; 80.7; 90.0; 87.8; 77.4 | 84.5 |
| 6031 | 0.1 | 89.8; 81.5; 90.3; 85.4; 94.7 | 88.4 |

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# A Kinetic Study of the Cyclization of L-Glutamine to 2-Pyrrolidone-5-carboxylic Acid in a Model System

The pseudo-first-order kinetics of the cyclization of glutamine to 2-pyrrolidone-5-carboxylic acid (PCA) and the one-half-order catalysis of this reaction by acetic acid were demonstrated in a model system.

The conversion of L-glutamine to the bitter tasting 2-pyrrolidone-5-carboxylic acid (PCA) in foods has been well documented (Foreman, 1914; Shallenberger et al., 1959; Mahdi et al., 1961; Clydesdale et al., 1972). Some of the effects of thermal processing, storage, and nitrogen fertilization on the formation of PCA in beets have also been studied by Lee et al. (1971). In addition, Archibald (1945) reported that complete conversion of glutamine to PCA was catalyzed by both acid and base. Wilson and Cannan (1937) examined the equilibrium between glutamic acid and PCA at pH values above 10 and below 4 using an indirect method of analysis for glutamic acid. The deamination and cyclization of glutamine to PCA are different from the glutamic acid–PCA equilibrium. Therefore, this study was undertaken to determine the effect of acid on the kinetics of the transformation of glutamine to PCA in the pH ranges of most foods (3–6). Acetic acid and sodium hydroxide were chosen as the buffer system for this study.

EXPERIMENTAL SECTION

**Materials.** All chemicals were reagent grade and obtained from commercial sources. The L-glutamine used had a specific optical rotation of $[\alpha]^{22}D$ +6.2° (1% $H_2O$).