Infrared Analysis for Residues of Isopropyl *N*-(3-Chlorophenyl)carbamate (CIPC) in White Potatoes

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It is necessary to possess a sensitive and specific method for determining the amount of CIPC which remains with potatoes after chemical treatment with CIPC for sprout inhibition. The infrared analytical method provides for these measurements and possesses a high degree of specificity for CIPC. Recovery of CIPC from fortified samples of potatoes is about 91% within the 2 to 20 p.p.m. concentration range. The method describes sample preparation, extraction of CIPC from the macerated sample, chromatographic cleanup of the residue, and infrared absorption measurement of the residue dissolved in carbon disulfide. This method is also applicable to other samples where interferences may be eliminated by column chromatography prior to infrared measurement.

ONE OF THE MOST SERIOUS PROBLEMS facing commercial potato processers and warehousemen is spoilage of the tubers due to sprouting while in storage or during transportation to market. To minimize this loss, a number of organic chemical compounds including isopropyl *N*-(3-chlorophenyl)carbamate (CIPC); 2, 4, 5-trichlorophenoxyacetic acid; maleic hydrazide; and the methyl ester of naphthaleneacetic acid have been examined for their effectiveness as sprout inhibitors for white potatoes.

Results of sprout inhibition experiments for potatoes involving these and other chemicals were reported by Marth and Schultz (*6*) and by Heinze *et al.* (*5*). These investigators reported that CIPC was a more effective sprout inhibitor for white potatoes than the other chemicals tested. In the course of their experimentation, several methods of applying the chemical to the tubers were explored, which included spraying of an aqueous emulsion, dipping in an aqueous emulsion, and vapor migration from paper and burlap sacks impregnated with CIPC.

As a result of these investigations and other experimental work conducted by the Pittsburgh Plate Glass Co., Chemical Division, a system of application of the chemical in the form of an aerosol was developed which proved especially effective in warehouse applications.

It became very important to possess a sensitive and specific analytical method for measuring the amount of CIPC remaining with the potatoes after chemical treatment and storage in order to meet tolerance requirements of the Food and Drug Administration. The infrared analytical method which has been developed provides these measurements and is specific for CIPC in the presence of similar chemicals such as 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron).

Analytical Methods

Analytical methods for determining residue concentrations of CIPC in white potatoes were described by Driver (*1*), and by Gard (*2*). The analytical method by Driver involves extraction of the macerated sample with *n*-hexane and ultraviolet absorption measurement directly on the extract to determine the CIPC content. Experimentally, this procedure presented technical complications during the extraction which resulted in the formation of stable emulsions which were difficult or impossible to break by ordinary means. Poor and inconsistent recovery values for CIPC, as well as high and unreliable blank or interference values for untreated tubers, were obtained by this procedure. In addition to these problems, the Driver method was not capable of distinguishing between monuron, diuron, and CIPC since the wavelengths for maximum absorbance in the ultraviolet region for the compounds are extremely close—monuron, 246 mμ; diuron, 248 mμ; CIPC, 239 mμ in methanol.

In 1959, Gard (*2*) reported the experimental results of analytical studies on potatoes treated by dipping or spraying the tubers with CIPC. This analytical procedure involves extraction of the chemical from a macerated sample with methylene dichloride, acidic hydrolysis of the CIPC in the evaporated extract to 3-chloroaniline, steam distillation of the 3-chloroaniline, and color development and spectrophotometric measurement employing the phenol-ammonia-hypochlorite method of Gard and Rudd (*3*). The most important limitation of this method is that it is not specific for CIPC because similar organic nitrogen-containing compounds, particularly monuron and diuron, also respond in a manner similar to CIPC.

Experimental Work

Preparation of the infrared absorption spectra of CIPC, monuron, and diuron in carbon disulfide solutions, as individual constituents and in combination, was the first operation in the development of the analytical method. These spectra are reproduced in Figure 1 and show that CIPC can be distinguished from monuron and diuron at spectral wave numbers of 1110 and 1210 cm.$^{-1}$, since these two chemicals register no absorbance in this spectral region. These spectra also reveal that wave numbers other than the aforementioned may, in special cases, be alternatively used for the CIPC analysis. Some spectral response for CIPC is registered at wave numbers of 675 and 765 cm.$^{-1}$. These responses possess less intensity than the major responses at 1110 and 1210 cm.$^{-1}$ and thus provide limited applicability. Another absorption band for CIPC at wave number 1740 cm.$^{-1}$ is nearly as strong as the major wave numbers, but fringes on the spectral region of 1685 cm.$^{-1}$, where monuron and diuron register. Use of this spectral region is limited because of potential overlap if high concentrations of monuron and diuron are involved in the same analysis.

Infrared Analytical Procedure

The infrared analytical procedure applicable to the analysis of white potatoes describes the necessary reagents, assembly of the special apparatus,

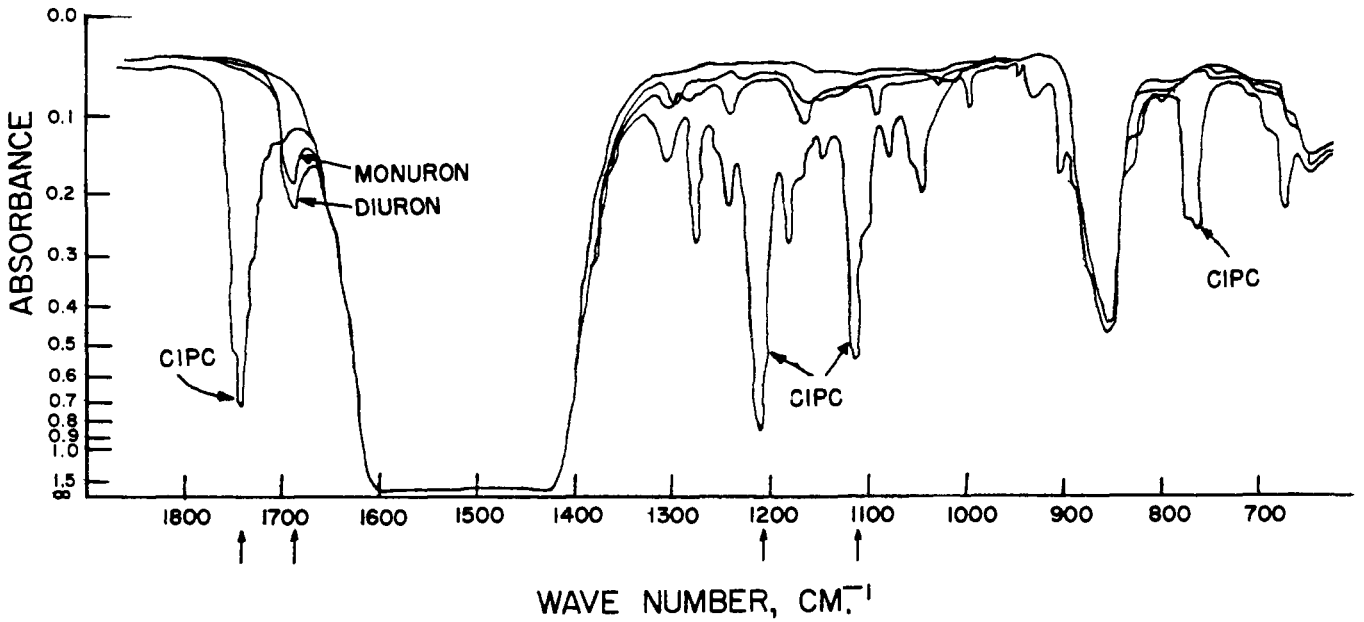

Figure 1. Infrared absorption spectra of CIPC, monuron, and diuron

sample preparation, extraction, chromatographic treatment of the residue, and the infrared absorption measurements.

Reagents. The reagents required for the analysis include methylene dichloride (reagent grade), carbon disulfide (reagent grade), and aluminum oxide (chromatographic grade).

Apparatus. The apparatus includes a Waring Blendor which accommodates a 1-quart blending jar, a centrifuge (International Equipment Co., Model SBR) which accommodates 250-ml. sedimentation bottles, a chromatographic column, and an infrared spectrophotometer (Perkin-Elmer Co., Model No. 21).

The chromatographic column is prepared in a 25-ml. straight buret by placing a loosely packed glass wool plug in the buret at the constriction near the bottom. Aluminum oxide is placed on top of the packing in an amount sufficient to give a bed height of 13 cm. Another glass wool plug which serves as a filter in eliminating solids from the solvent is placed on the top surface of the aluminum oxide.

Sampling. Treated potatoes stored in the warehouse are sampled by selecting some 10 to 12 average-sized representative tubers from which the analytical sample is prepared. Without washing, peeling, or scrubbing, each tuber is cut into eight segments by a system of halving and quartering. A sufficient number of segments from each tuber is selected to constitute 300 to 400 grams.

Maceration and Extraction of Samples. The selected segments are cut into small pieces, then placed in the mixing jar of the Waring Blendor, and macerated to a fine pulp without the addition of water or solvent. Two hundred grams of the macerate is weighed into a beaker, then transferred to a 500-ml. separatory funnel, using a minimum amount of water to effect quantitative transfer. Two hundred milliliters of methylene dichloride is added to the weighed macerate and shaken vigorously for 2 minutes, then set aside and digested for 10 minutes to aid in dissolving and extracting the CIPC from the pulp. Separation of the various layers is not voluntary, and the use of a centrifuge is required. The pulp–methylene dichloride mixture is divided evenly between two 250-ml. sedimentation bottles, and centrifuged for 15 minutes at 1800 r.p.m. This operation divides the sample mixture into three distinct layers—the top layer, which is basically water; the middle layer, which is potato pulp; and the bottom layer, which is methylene dichloride containing the CIPC in solution.

The separation and transfer of the layers resulting from centrifugation must be conducted very carefully to prevent mechanical loss of CIPC. The aqueous layer is withdrawn from the bottles with the aid of a suction bulb and returned to the original separatory funnel for further treatment. The semirigid pulp layer is carefully punctured with a stirring rod and the methylene dichloride phase poured from the bottle through a glass wool plug contained in the vertex of a funnel and collected in a 500-ml. Erlenmeyer flask equipped with a standard taper glass joint and stopper. A considerable amount of the residual methylene dichloride in the pulp is removed by mashing and squeezing with a stirring rod or spatula. As much of the pulp as possible is retained in the sedimentation bottle during this operation. Similar operations are conducted for both sedimentation bottles, and the methylene dichloride extracts are combined in the Erlenmeyer flask.

After this separation, the pulp is returned quantitatively to the original separatory funnel and re-extracted with additional 200-ml. portions of methylene dichloride. A total of three extractions, centrifugations, and separations are conducted prior to discarding the aqueous and pulp layers of the sample.

Application of the method is expedited by commencing the evaporation of the filtered methylene dichloride extract as soon as the first portions are collected. This provides space in the flask for solvent derived from subsequent extractions and permits evaporative operations to proceed during the re-extraction operations.

Solvent evaporation is conducted at reduced pressure by attaching the flask to a water aspirator pump adjusted to permit even boiling of the solvent when the flask is warmed to about 40° C. in a water bath. Evaporation is allowed to proceed until a volume of about 15 ml. is reached. As filtered solvent fractions from subsequent extractions become available, they are added to the flask and evaporated.

The last portion of solvent is evaporated quite carefully, and when approximately 5 ml. remains, the flask is withdrawn from the heated environment, and evaporation to apparent dryness is continued at reduced pressure only. It is not necessary or desirable to evaporate to absolute dryness, but only until traces of solvent remain.

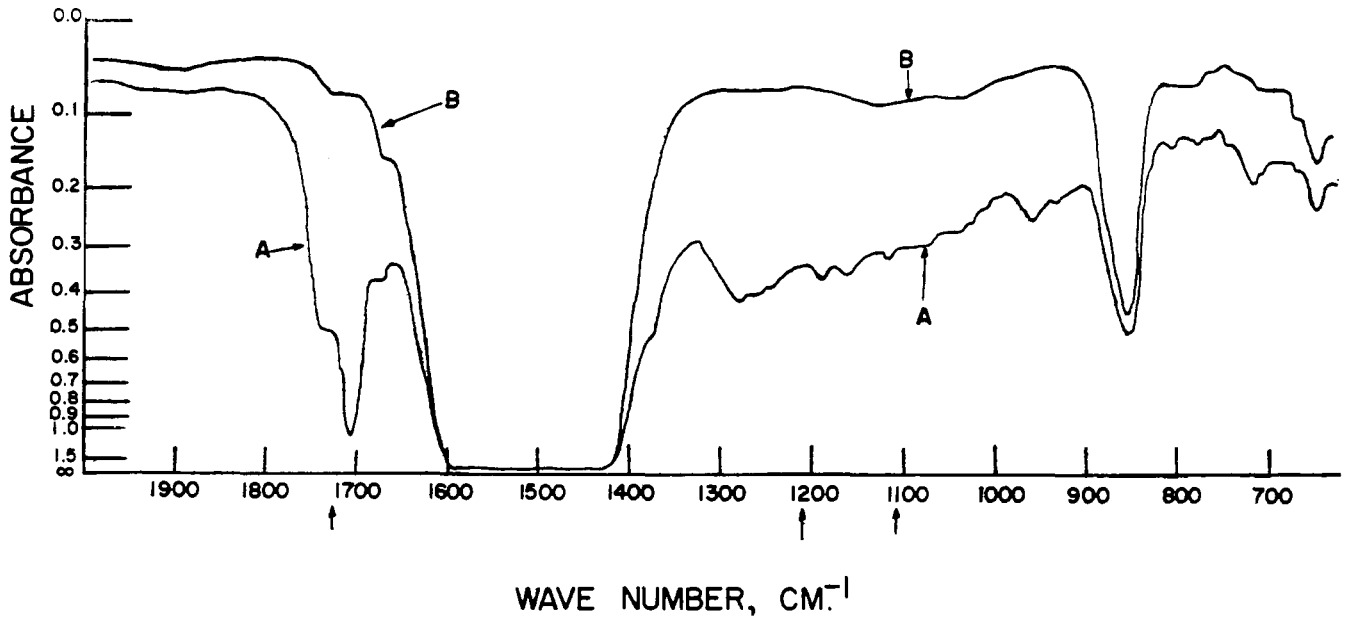

Figure 2. Infrared absorption spectra of potato extract before (*A*) and after (*B*) chromatography

Chromatographic Cleanup of Residue. Some of the inherent interferences due to unknown constituents in potatoes are removed by chromatographing the extract through an aluminum oxide bed. This operation is conducted by treating the residue remaining in the flask with 5 ml. of methylene dichloride to dissolve the soluble constituents, including CIPC. Solution of these constituents is expedited by swirling the flask occasionally during a 5-minute digestion period.

A clean 100-ml. flask is positioned at the exhaust of the column to catch the effluent. The solution of the residue is carefully poured onto the prepared chromatographic column and is permitted to percolate through the aluminum oxide bed. The flask is rinsed with a 2-ml. portion of the methylene dichloride, adding the rinsings to the chromatographic column when the liquid level in the column reaches the top surface of the glass wool plug. This rinsing procedure is conducted two additional times to effect quantitative transfer of the dissolved residue to the column. When the level of liquid from the washings reaches the glass wool plug, additional methylene dichloride is added incrementally and in sufficient amounts to provide for the collection of 75 ml. of effluent from the column in the receiver flask.

After chromatography, the collected effluent is again evaporated carefully to apparent dryness under conditions of reduced pressure and gentle heating in the manner described previously for the solvent before chromatography. This evaporative operation must be done very carefully to minimize CIPC losses, since CIPC possesses an appreciable vapor pressure and losses may result if overheating or prolonged evaporation at reduced pressure is permitted.

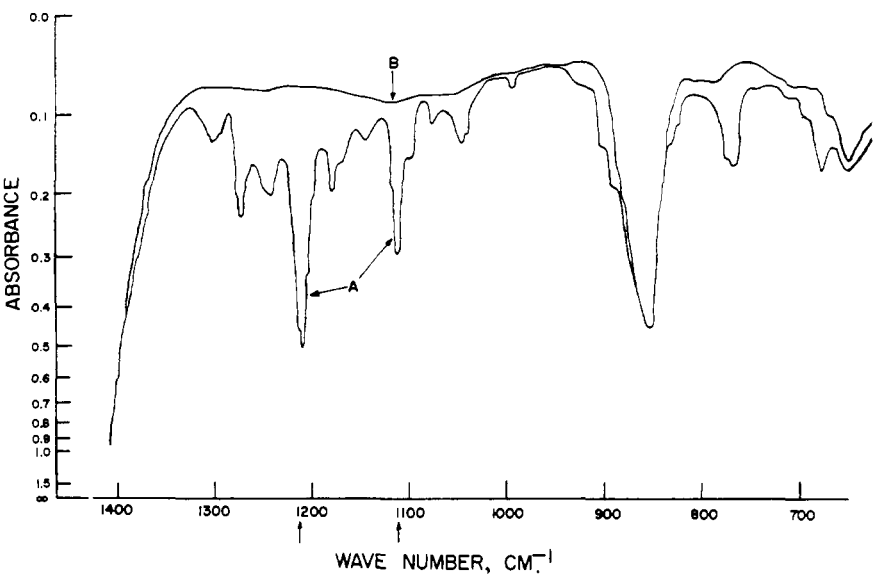

Figure 3. Infrared absorption spectra of potato extract with (*A*) and without (*B*) CIPC

Infrared Absorption Measurement. The residue remaining in the flask after chromatographing is treated with 5.0 ml. of carbon disulfide, and the flask is closed by crimping a small piece of aluminum foil over its top. The flask is swirled intermittently during a 5-minute period to effect the solution of the residue. When solution is complete, the aluminum foil closure is removed, and a sufficient amount of sample is withdrawn with a hypodermic syringe to flush and fill a 0.5-mm. fixed thickness absorption cell fitted with sodium chloride windows.

The spectrum of the solution is recorded between the wave numbers of 1250 and 1050 cm.$^{-1}$ using appropriate conditions for obtaining quantitative spectra, and employing sodium chloride compensation in the reference beam of the spectrophotometer.

The CIPC in the extract is calculated from the recorded spectra using the system of baseline compensation described by Heigl *et al.* (*4*). The baseline corrected absorbances are recorded directly from the chart for the two CIPC bands at 1210 and 1110 cm.$^{-1}$ as the absorbance difference between the baseline and the absorption maximum at these frequencies.

Calculation. The concentration of CIPC in the potato sample is computed as follows:

$$\text{p.p.m. CIPC} = \frac{A_{B_1} \times V \times 1000}{a_1 \times S}$$

where

A_{B_1} = baseline corrected sample absorbance at the frequency of the absorption maximum.
a_1 = absorbance index at the same frequency.
S = grams of sample extracted (200 grams).
V = volume of carbon disulfide used in dissolving residue (5 ml.).

Calibration of Infrared Spectrophotometer

CIPC, specially purified by recrystallization from hexane, is used for calibrating the infrared spectrophotometer. Calibration is conducted by preparing solutions of varying concentrations of CIPC in carbon disulfide and measuring the infrared absorbance as related to the concentration.

Exactly 0.200 gram of the purified CIPC is weighed and dissolved in carbon disulfide and diluted to 100 ml. in a volumetric flask. Measured portions (10.0, 20.0, and 50 ml.) of this standard solution are further diluted to 100 ml. with carbon disulfide. The resulting standard solutions contain 0.2, 0.4, 1.0, and 2.0 grams of CIPC per liter of solution.

The 0.5-mm. absorption cell is filled with the standard solutions, and their respective absorbance values are recorded and measured in the spectral range of 1250 to 1050 cm.$^{-1}$ employing the same instrument operating conditions used for sample measurement. The baseline corrected absorbances of each of the standards are measured and recorded at wave numbers 1210 and 1110 cm.$^{-1}$, which are the analytical spectral bands used in the method.

Absorbance indices are calculated for each standard at each of the two wave numbers by dividing the absorbances of the standards by their concentrations. If for any reason the separate indices thus calculated for the four standards at either wave number differ by more than 10%, plot absorbance values *vs.* concentration directly to provide an empirical calibration curve for the instrument.

Analytical Results

Figure 2 shows the infrared absorption spectra obtained from the extract of a typical untreated white potato sample both before and after use of the chromatographic cleanup step. These spectra show the presence of substantial amounts of interferences in the potatoes which are extracted by the methylene dichloride and are removed by this treatment. These interferences appear to be common to all types of white potatoes since they were observed in the spectra of extracts from untreated potatoes from many different sources. Potatoes from Ohio, Nebraska, Maine, Long Island, Idaho, Pennsylvania, and California, as well as Katahdin, Kennebec, Redskin, Idaho, and Irish Cobblers, all exhibited similar infrared spectra, and are capable of cleanup by this chromatographic procedure.

Figure 3 shows the infrared spectra of a chromatographed extract from a typical untreated potato sample as compared with a similar extract fortified in the laboratory with CIPC. The sharp absorption peaks for CIPC at 1110 and 1210 cm.$^{-1}$ after chromatographic cleanup are outstanding and are easily distinguished from the untreated extract.

Recovery of CIPC. At the onset of the experimental work, recovery analyses were conducted to determine the accuracy of the analytical method. The results of the recovery analysis are given in Table I.

Table I. Recovery of Isopropyl *N*-(3-Chlorophenyl)carbamate (CIPC) Added to White Potatoes

Added CIPC, P.P.M.	Found CIPC, P.P.M.	Recovery, %
20	16.3	81.5
10	9.6	96
2	1.8	90
5	4.8	96
10	10	100
15	14.3	95.3
10	7.6	76
3.8[a]	3.6	94.7
	Av.	91.2

[a] This sample was a treated sample, with the value 3.8 p.p.m. representing the average of five determinations by the spectrophotometric method (3).

Related Analytical Applications

This infrared analytical method is applicable to the measurement of small amounts of CIPC in various materials where solvent extraction procedures perform satisfactorily and nominal interference values are observed in the infrared region.

One application of the analytical method in this laboratory was the analysis of wood panels from various storage bins, crates, and packing containers used for treated potatoes. Examination of these wooden specimens for CIPC was a part of a cooperative experimental program conducted by H. A. Murphy and H. V. Toko, United States Department of Agriculture, Agricultural Marketing Service, Presque Isle, Maine. The purpose of this program was to determine any accumulation of CIPC resulting from multiple exposure to the chemical. Samples of wood panels of various types, such as fir, spruce, hemlock, plywood, and others, were washed with solvent, and the extracts analyzed for CIPC by the infrared analytical method.

Another important and successful application of the method was its use in determining the active herbicidal ingredient (CIPC) in various commercial formulations of liquid and granular products.

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