products. Though the lower detection limits of this HPLC methodology do not compare favorably with the more sensitive capillary GC analysis (Maerker and Unruh, 1986), the HPLC method is particularly effective when the need arises to separate and measure cholesterol oxides that are sensitive to the elevated temperatures of GC (Maerker, 1987) and that are unresolvable by TLC.

ACKNOWLEDGMENT

We thank Dr. John G. Phillips for helpful advice concerning the statistical evaluation of data.

Registry No. Cholestra-3,5-dien-7-one, 567-72-6; cholesterol, 57-88-5; cholest-5-ene-3β,25-diol, 2140-46-7; cholesterol 5α,6α-epoxide, 1250-95-9; cholesterol 5β,6β-epoxide, 4025-59-6; 5α-cholestan-6-one, 570-46-7; 3β-hydroxycholest-5-en-7-one, 566-28-9; cholest-5-ene-3β,7β-diol, 566-27-8; cholest-5-ene-3β,7α-diol, 566-26-7; 5α-cholestane-3β,5,6β-triol, 1253-84-5.

LITERATURE CITED

Addis, P. B.; Csallany, A. S.; Kindom, S. E. *ACS Symposium Series*; Finley, J. W., Schwass, D. E., Eds.; American Chemical Society: Washington, DC, 1983; No. 234, pp 85–98.
Ansari, G. A. S.; Smith, L. L. *J. Chromatogr.* **1979**, *175*, 307–315.
Bascoul, J.; Domergue, N.; Crastes de Paulet, A. *Steroid Biochem.* **1983**, *19*, 1779–1782.
Bascoul, J.; Domergue, N.; Ole, M.; Crastes de Paulet, A. *Lipids* **1986**, *21*, 383–387.
Chicoye, E.; Powrie, W. D.; Fennema, O. *Lipids* **1968**, *3*, 335–339.
Csiky, I. *J. Chromatogr.* **1982**, *241*, 381–389.
Davis, M.; Petrow, V. A. *J. Chem. Soc.* **1949**, 2536–2539.
Fieser, L. F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1963**, *71*, 3938–3941.
Finocchiaro, E. T.; Lee, K.; Richardson, T. *JAOCS, J. Am. Oil Chem. Soc.* **1984**, *61*, 877–883.
Maerker, G. *JAOCS, J. Am. Oil Chem. Soc.* **1987**, *64*, 388–392.
Maerker, G.; Unruh, J., Jr. *JAOCS, J. Am. Oil Chem. Soc.* **1986**, *63*, 767–771.
Maerker, G.; Bunick, F. J. *JAOCS, J. Am. Oil Chem. Soc.* **1986**, *63*, 771–777.
Missler, S. R.; Wasilchuk, B. A.; Merritt, C., Jr. *J. Food Sci.* **1985**, *50*, 595–598, 646.
Norman, H. A.; St. John, J. B. *J. Lipid Res.* **1986**, *27*, 1104–1107.
Park, S. W.; Addis, P. B. *J. Food Sci.* **1985**, *50*, 1437–1441, 1444.
Peng, S.-K.; Taylor, C. B. In *World Review of Nutrition and Dietetics*; Bourne, G. H., Ed.; S. Karger: Basel, Switzerland, 1984; Vol. 44, pp 117–154.
Peng, S.-K.; Taylor, C. B.; Hill, J. C.; Morin, R. J. *Atherosclerosis* **1985**, *54*, 121–133.
Phillips, F. C.; Erdahl, W. L.; Nadenicek, J. D.; Nutter, L. J.; Schmit, J. A.; Privett, O. S. *Lipids* **1984**, *19*, 142–150.
Shen, C.-S. J.; Sheppard, A. J. *Chromatographia* **1983**, *17*, 469–471.
Smith, L. L. In *Cholesterol Autoxidation*; Plenum: New York, 1981.
Smith, L. A.; Norman, H. A.; Cho, S. H.; Thompson, G. A., Jr. *J. Chromatogr.* **1985**, *346*, 291–299.
Sugino, K.; Terao, J.; Murakami, H.; Matsushita, S. *J. Agric. Food Chem.* **1986**, *34*, 36–39.
Tsai, L. S.; Hudson, C. A. *JAOCS, J. Am. Oil Chem. Soc.* **1981**, *58*, 931–934.
Tsai, L. S.; Hudson, C. A. *J. Food Sci.* **1985**, *50*, 229–231, 237.
Tsai, L. S.; Ijichi, K.; Hudson, C. A.; Meehan, J. J. *Lipids* **1980**, *15*, 124–128.

Received for review March 30, 1987. Revised manuscript received June 25, 1987. Accepted August 11, 1987.

Analysis of Chlorimuron Ethyl in Crops by High-Performance Liquid Chromatography

James L. Prince and Richard A. Guinivan*

Methods are presented for the residual determination of chlorimuron ethyl in soybeans and some soybean rotational crops (wheat, corn kernels, potatoes, turnips). Chlorimuron ethyl was extracted from the matrix and cleaned up by one or two of four available methods (a doublet Bond Elut Si column, Sep Paks, a LiChroprep Si60 medium-pressure liquid chromatography column, or aqueous–organic solvent partitioning). The compound was then quantitated in a normal-phase HPLC employing a photoconductivity detector. The minimum detection level was 0.01 ppm as established with fortification recovery experiments, and recoveries averaged above 90% for 0.01 to 0.1 ppm fortifications.

Chlorimuron ethyl (Figure 1) is the active ingredient of a new selective soybean herbicide sold by Du Pont under the trade name of CLASSIC herbicide. This molecule belongs to a class of compounds called sufonylureas. These compounds in general are thermally unstable, rendering them difficult to detect by gas chromatography procedures; however, several investigators have made sulfonylurea derivatives detectable by gas chromatography (Braselton et al., 1975, 1976, 1977; Midha et al., 1976; Kleber et al., 1977; Prescott and Redman, 1972; Sabih and Sabih, 1970; Simmons et al., 1972; Hartvig et al., 1980). Other investigators used liquid chromatography with ultraviolet absorbance detectors to analyze for these compounds (Beyer, 1972; Harzer, 1980; Molins et al., 1975; Raghow and Meyer, 1981; Reinaure et al., 1980; Robertson et al., 1979; Sved et al., 1976; Tsugi and Binns, 1982; Uihlein and Sistovaris, 1982; Waahlin-Boll and Melander, 1979; Weber, 1976). Kimura et al. (1980) compared gas chromatography and liquid chromatography methods and found similar sensitivities and reproducibilities.

Some other approaches used for sulfonylurea analyses include radioimmunoassay (Kajinuma et al., 1982; Kelley et al., 1985), bioassay (Hsiao and Smith, 1983; Bond and Roberts, 1976), hydrolysis followed by fluorescence detection of the dansyl derivative (Huck, 1978), and precolumn derivatization followed by fluormetric detection (Besenfelder, 1981).

Recent approaches at analyzing pesticides, including sulfonylureas, have made use of the liquid chromatography, photoconductivity detector. This detector is sensitive and selective for sulfur, halogens, nitrogen, and phosphorus atoms. Studies using this detector for pesticides have been reported by Buttler and Hormann (1981), Walters (1983),

Agricultural Products Department, Research Division, E. I. du Pont de Nemours and Company, Inc., Experimental Station, Wilmington, Delaware 19898.

0021-8561/88/1436-0063$01.50/0

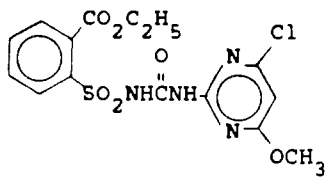

Figure 1. Chlorimuron ethyl. Benzoic acid, 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]-, ethyl ester.

and Walters and Gilvydis (1983). Methods using the photoconductivity detector for analyses of sulfonylurea herbicides include the work of Zahnow (1982, 1985a,b, 1986) and Slates (1983).

For the analysis of chlorimuron ethyl three cleanup methods all followed by liquid chromatography quantitation using the photoconductivity detector have been developed. Two of the cleanup methods are used for the analysis of residues of this compound in soybeans. Method 1 involves the extraction of chlorimuron ethyl from 25 g of soybean into methylene chloride followed by a hexane–aqueous partitioning. The chlorimuron ethyl was then removed from the water into methylene chloride and cleaned up on a doublet Bond Elut Si column. Method 2 involves the extraction of chlorimuron ethyl from 10 g of soybeans into ethyl acetate followed by cleanup on a LiChroprep Si60 medium-pressure liquid chromatography column using ethyl acetate–methanol as the eluent. This second method can also be used for the analysis of chlorimuron ethyl in wheat and corn kernels.

Ethyl acetate extraction will also remove chlorimuron ethyl from turnips and potatoes, but these extracts cannot be cleaned up on silica since the compound will not elute from the column when applied in turnip and potato extracts. However, the chlorimuron ethyl remains in a basic aqueous buffer when the buffer is extracted with hexane and ethyl acetate. As a result, many interferences are removed by solvent extraction in this manner. When the pH was adjusted to 2.5, the chlorimuron ethyl was removed from the aqueous phase with ethyl acetate and then purified further on a silica (turnips) or C-18 (potatoes) Sep-Pak. The pH manipulation of solvent extractability combined with a Sep-Pak cleanup comprises method 3.

Chlorimuron ethyl is quantitated in all cases by normal-phase HPLC using a photoconductivity detector. This detector, which measures the difference in conductivity between UV-exposed and nonexposed LC column effluent, allows for both selectivity and sensitivity.

EXPERIMENTAL SECTION

Materials. *Chlorimuron ethyl reference standard*: supplied by Du Pont Agricultural Products Department, Wilmington, DE.

Solvents: distilled-in-glass grade hexane, acetone, 2-propanol, methanol, methylene chloride, and ethyl acetate, supplied by Burdick and Jackson, Muskegon, MI, Baker Chemicals, Phillipsburg, NJ, or Fisher, Philadelphia, PA. The solvents were filtered through a 0.5-mm type FH Millipore filter (Millipore Corp., Bedford, MA) before use to ensure removal of all fine particulate matter.

Grinding mill: Model 4E from Quaker City Mill, Philadelphia, PA.

Flash evaporator: Rotovapor-R from Brinkmann Instruments, Westbury, NY, operated at 26 in. of mercury vacuum with the flask in a water bath operated between 35 and 50 °C.

Nitrogen evaporator: Meyer N-EVAP evaporator, Model 111, from Organomation Associates, Inc., Northborough, MA.

Centrifuges: desk top International Clinical Centrifuge and the IEC Model K centrifuge from International Equipment Co., Needham Heights, MA.

Sylon CT: 5% dimethyldichlorosilane in toluene from Supelco., Inc., Bellefonte, PA.

Vortex-Genie: Scientific Industries, Inc., Bohemia, NY.

Test tubes: Kimble, Kimax, 13 × 100 mm culture tubes with Teflon rubber-lined screw caps. Kimax 15- and 50-mL graduated centrifuge tubes were used for volume reduction.

Homogenizer: Tekmar SKT Tissumizer equipped with an SDT-182 EN shaft and generator from Tekmar Co., Cincinnati, OH.

Precision pipetting system: Pipetman from Rainin Instrument Co., Inc., Woburn, MA.

Silica Sep-Pak: Waters Associates, Milford, MA (Catalog No. 51900).

Silica Bond Elut: Analytichem International, Harbor City, CA (Catalog No. 601303).

Bond Elut adaptor: Analytichem International, Harbor City, CA (Catalog No. 636001).

Medium-pressure silica gel column: LiChroprep Si 60 (40–63 μm) silica gel column (310 mm × 25 mm) from EM Laboratories, Inc., Elmsford, NY. The column was equilibrated by pumping 60% ethyl acetate–40% methanol through the column for 3 h at a flow rate of 8 mL/min with a Milton Roy minipump. The operational flow rate was also 8 mL/min. A Rheodyne valve with a 1-mL loop was used to apply the sample.

Liquid chromatograph: basic system used in these studies was a Du Pont 8800 series liquid chromatographic column compartment (PN 851100-901), pump (PN 861006-000), and controller (PN 861306-900) supplied by Du Pont, Analytical Instruments Division, Wilmington, DE. The chromatograph was equipped with a Tracor 965 photoconductivity detector, a Perkin-Elmer, 1-mV R-100 recorder, and either a 25.0 cm × 4.6 mm Du Pont Zorbax SIL column or a Waters μ-Porasil (3.9 mm × 30 cm) column from Millipore Waters Chromatography Division, Milford, MA. The mobile phase was 75% hexane–12.5% propanol–12.5% methanol with 2 mL of acetic acid and 1 mL of water added/L of mobile phase. The flow rate was 1 mL/min, and the oven compartment was maintained at 35 °C. Under these conditions chlorimuron ethyl elutes at 5–6 min.

Liquid Chromatography. The photoconductivity detector (Tracor Model 965) was used at a sensitivity of 1 × 50 (25-g samples) or 1 × 20 (10-g samples) to achieve the desired lower detection level. Therefore, it was essential that the chromatographic system provide good temperature control of the column and reasonably pulse-free delivery of mobile phase to minimize base-line fluctuations.

The photoconductivity detector was used for this analysis to obtain adequate sensitivity and selectivity. The mercury lamp was used in the detector since it provided much greater sensitivity than the zinc lamp. The detector, including the lamp, was left on at all times to ensure greater stability. The flow of the mobile phase through the reference and analytical loops was balanced to within ±5%. This was accomplished by installing a metering valve in the solvent line that exits from the reference compartment of the conductivity cell. The "T" that brought the two solvent lines from the conductivity cell back together was eliminated from the instrument. Also, the ion-exchange resin tube was not needed to pufify the mobile phase and could actually introduce unwanted materials into the system.

New columns must be conditioned by pumping a conditioning solution (10 parts by volume of 2-propanol, 10 of methanol, 5 of glacial acetic acid, and 1 of water) through it for several hours at 1 mL/min. This treatment was also used to clean columns that started to lose their efficiency because of contamination from samples. A contaminated column was characterized by broad peaks that tailed very badly and by shifting retention times. This conditioning solvent was thoroughly flushed from the column with the mobile phase. Flushing for 1 h at 1.0 mL/min was usually sufficient.

A sample valve (20 μL for method 1 and 10 μL for methods 2 and 3) was used for manual injection of standards and samples, to minimize contamination of the HPLC column and broaden the chromatographic peaks.

Silanization of Glassware. Test tubes, glass pipets, and evaporation flasks may need to be silanized if recoveries are low (consistently below 75%). This tends to occur when glassware is used and conditioned to analyses other than chlorimuron ethyl. The Sylon CT solution was simply shaken in contact with the glassware surface for 1.0 min and then rinsed with toluene and methanol. Just enough Sylon CT to ensure good surface contact was needed. Recoveries could be reduced by ~30% if the glass is not occassionally deactivated. The glassware needed to be retreated when recoveries started to drop below 80% consistently.

METHODS

The next two sections present methods for analysis of fortified soybean seed, wheat grain, corn, turnips, and potatoes. These studies are intended to be representative of the analysis requirements on the majority of similar crop varieties.

Soybeans. Method 1. Approximately 100 g of soybeans was ground in a Waring blender to a fine powder and mixed thoroughly. A 25 ± 0.1 g sample of ground soybean was placed into a 250-mL centrifuge bottle, a 100-mL volume of methylene chloride was added, and the mixture was homogenized for 2 min at maximum speed by the Tekmar Tissumizer. This slurry was filtered through a Buchner funnel containing a No. 42 filter paper into a 500-mL round-bottom flask. The Buchner funnel was attached to the 500-mL round-bottom flask by means of a glass adaptor containing a side arm attached to a vacuum source. The matrix was removed from the funnel and returned to the centrifuge bottle. An additional 100 mL of methylene chloride was added to the centrifuge bottle, and the bottle was swirled to mix the contents. This mixture was filtered through the Buchner funnel into the 500-mL round-bottom flask. The 500-mL round-bottom flask was disconnected from the Buchner funnel, and 100 mL of deionized water was added to the solution in the round-bottom flask. This flask was then attached to a flash evaporator and placed in a water bath at 50 °C and the methylene chloride removed under vacuum. The remaining aqueous solution, after the methylene chloride was removed, was quantitatively transferred to a 250-mL separatory funnel. The round-bottom flask was washed with an additional 25 mL of water that was added to the separatory funnel. The aqueous phase was washed three times with 100 mL of hexane each time. The first wash used gentle shaking (approximately 1 min) to avoid serious emulsions. The aqueous layer (lower layer) was drained into a second 250-mL separatory funnel and the hexane discarded. The second and third 100 mL of hexane was added and shaken for 1 min. The solution was allowed to stand to obtain clear separation of the two phases. The aqueous layer (lower layer) was drained back into the first 250-mL separatory funnel and the hexane discarded. The aqueous phase was then extracted three times with 50 mL of methylene chloride. Each extraction was shaken for 1 min, and the layers were allowed to separate. The methylene chloride (lower layer) was drained, after each extraction, through 5 g of sodium sulfate and cotton contained in a 7.5-cm glass funnel into a 250-mL round-bottom flask. The methylene chloride was then evaporated to approximately 5 mL under vacuum on the flash evaporator with the flask in a water bath maintained at 50 °C.

Two Bond Elut Sil cartridges were connected in series by a Bond Elut adaptor. A 15-mL Kimble centrifuge tube was placed in a 250-mL filter flask. The flask was attached to a vacuum source at the side arm. A 4-in., 16-gauge leur nut stainless-steel needle was inserted through a No. 6 rubber stopper and the stopper placed on the filter flask, with the needle extending into the Kimble centrifuge tube. The Bond Elut cartridges were fitted to the leur hub of the needle, and the column was washed with 5 mL of methylene chloride to condition the column. The 5-mL sample was transferred from the 250-mL round-bottom flask to the column with use of a Pasteur pipet. The 250-mL flask was then rinsed with 3–4 mL of methylene chloride and the rinse added to the column, allowing the rinse to pass through the columns. A small amount of vacum was sometimes necessary to maintain a flow rate of approximately 0.5 mL/min. The methylene chloride was discarded. The chlorimuron ethyl was eluted from the column with 10 mL of a mixture containing 85 parts by volume of cyclohexane, 10 isopropanol, and 5 of methanol, and the column eluent was collected in a second 15-mL Kimble tube. This solution was evaporated to dryness with a gentle stream of nitrogen on a nitrogen evaporator at a temperature of 50 °C. The sample was stored dry in a refrigerator until it could be analyzed. At the time of analysis, the sample was dissolved in mobile phase (see chromatography section) and diluted to a final volume of 1 mL. The entire sample was filtered through a Millex-SR 0.5 filter unit mounted on a 1-mL hypodermic syringe into a small vial prior to analysis, discarding the filter after each use.

Soybeans, Wheat, and Corn Kernels. Method 2. Soybeans and corn samples (~100 g) were passed through the grinding mill twice immediately upon removal from the freezer. A 10-g sample (ground soybean seed, wheat grain, or ground corn kernels) was placed in a 250-mL polypropylene centrifuge bottle, and a 200-mL volume of ethyl acetate was added before the sample was homogenized for 2 min with the Tekmar Tissumizer. The sample was then spun at full speed in the Model K centrifuge (4725*g*) for 10 min after which the supernatant was poured through a Schleicher & Schuell Grade 520B 1/2 prepleated filter paper and collected in a 1000-mL flask suitable for flash evaporation. A second 200-mL volume of ethyl acetate was added to the bottle, and the homogenization, centrifugation, and collection steps were repeated.

The combined supernatants were concentrated to ~3 mL by rotary evaporation in a 35 °C water bath and moved quantitatively to a 50-mL test tube with ethyl acetate rinses and a Pasteur pipet for transfers. The solvent was then evaporated with a stream of nitrogen flow, leaving an oil. This oil was diluted to 5 mL with 60% ethyl acetate–40% methanol, and the sample was passed through a 10-mL syringe mounted to a doublet silica Sep-Pak (two Sep-Paks joined by a polypropylene disposable pipet tip as a connector). The doublet was prewashed with 10 mL of 60% ethyl acetate–40% methanol before use. The syringe plunger was used to apply gentle pressure needed

to sequentially elute two, 1-mL rinses of the sample tube and a 7-mL wash (60% ethyl acetate–40% methanol) through the doublet in a dropwise fashion. All the eluants were collected in a 15-mL centrifuge tube. The solvent was evaporated to dryness under a stream of nitrogen (no water bath used). Following the above concentration step, considerable oil still remained in the samples. In general, soybeans had ~1.5–2.0 mL of oil, while corn and wheat grain samples had ~200 μL of oil remaining, after the solvent was evaporated.

The sample was then cleaned up with the silica medium-pressure liquid chromatography step. The sample volume was adjusted to 2.0 mL, if needed, with 60% ethyl acetate–40% methanol in the 15-mL tube. Since solids often form on concentration, the sample required a 10-min centrifugation in the desk top centrifuge to pelletize these particulates. The 2-mL sample was then used to fill the 1-mL loop on the medium-pressure LC silica LiChroprep column. Since only half the sample is applied to the column, the actual amount of crop analyzed was reduced from 10 to 5.0 g. When the sample was put in the syringe before loading the loop, a plug of air was drawn into the syringe by withdrawing the plunger after the 2.0-mL sample had been drawn in. The air plug traveled through the loop in front of the sample, separating it from mobile phase in the loop. Laminar flow disruptions were reduced with the air plug. After the loop was loaded, the valve was switched to the inject position and a 400-mL volume of eluant was discarded before 200 mL was collected in a flash evaporator flask and taken to dryness by rotary evaporation in a 35 °C water bath. The sample was quantitatively transferred from the evaporator flask to a culture tube with 3 × 2 mL ethyl acetate rinses. Transfers were done with a Pasteur pipet. The samples in the culture tube were blown to dryness under a stream of nitrogen (no water bath). The sample was then redissolved in 1 mL of HPLC mobile phase and analyzed by HPLC. Standards were prepared by aliquoting amounts (0.015–0.1 μg) from a 1 μg/mL stock in ethyl acetate into culture tubes. The solvent was blown off the standard aliquot under a stream of nitrogen (no water bath) and the standard redissolved in 1 mL of HPLC mobile phase.

Turnips and Potatoes. Method 3. All turnips in a sample to be analyzed were first cut into small pieces with a knife, and the pieces were well mixed. All potatoes in a sample were cut into eighths. An eighth from each potato was cut into small pieces with a knife, and these pieces were well mixed. A 10-g crop sample (turnip or potato) was placed in a 250-mL polypropylene centrifuge bottle. Ethyl acetate (200 mL) was added, and each sample was homogenized for 2 min with the Tekmar Tissumizer. The samples were spun at full speed in the Model K Centrifuge (4725*g*) for 10 min, and the supernatant was decanted into a flash evaporator flask. A second 200-mL volume of ethyl acetate was added, and the homogenization, centrifugation, and collection steps were repeated. The ethyl acetate was removed by rotary evaporation.

The samples were then transferred with ethyl acetate rinses from the flash evaporator flasks to 250-mL polypropylene centrifuge bottles containing 50 mL of 0.05 M sodium bicarbonate (adjusted to pH 10.0 with NaOH). The ethyl acetate was removed under a stream of nitrogen. A 100-mL volume of hexane was added to each bottle, and the sample and hexane were mixed for 1 min with the Tekmar Tissumizer. When the layers separated, the upper hexane layer was removed with a 50-mL syringe and discarded. Then, a 100-mL volume of ethyl acetate was added to each sample, and the sample and ethyl acetate were mixed for 1 min with the Tekmar Tissumizer. When the layers separated, the upper ethyl acetate layer was removed and discarded. The aqueous layer was adjusted to pH 2.5 with 6 N HCl. A 100-mL volume of ethyl acetate was added and mixed with the aqueous layer for 1 min (Tissumizer), and then the upper ethyl acetate layer was removed and placed in a flash evaporator flask. A second 100 mL of ethyl acetate was added to the aqueous fraction, and the homogenization and collection steps were repeated. The ethyl acetate was removed with rotary evaporation in a 35 °C water bath, and the samples were quantitatively transferred with a Pasteur pipet to 15-mL centrifuge tubes with ethyl acetate rinses. The samples were taken to dryness under a stream of nitrogen (no water bath).

The potato samples were dissolved in 4 mL of 0.05 M sodium bicarbonate (adjusted to pH 11.0 with NaOH). A 1-mL sample was removed with a precision pipettor and applied directly to a 10-mL syringe mounted on a C-18 Sep-Pak that was prewashed (10 mL of methanol followed by 8 mL of pH 11.0 bicarbonate buffer). A 4-mL volume of pH 11.0 bicarbonate buffer was then passed through the Sep-Pak and the eluant discarded. A 10-mL volume of 30% acetonitrile–70% bicarbonate buffer was then put through the Sep-Pak and the effluent collected in a 50-mL test tube. The pH of the effluent was changed to ~2.5 with the addition of 6 drops of 6 N HCl. A 10-mL volume of ethyl acetate was added to the 50-mL tube, the tube contents were mixed for 1 min on the Vortex-Genie, and the upper ethyl acetate layer was removed with a Pasteur pipet and placed in a 50-mL test tube. Another 10 mL of ethyl acetate was added to the 50-mL effluent tube, and the mixing and collection steps were repeated. The combined ethyl acetate fractions were taken to dryness under a stream of nitrogen (no water bath), redissolved in LC mobile phase, and analyzed by HPLC. Standards were prepared as for procedure 2.

The turnip samples were dissolved in 4 mL of HPLC mobile phase. A 1 mL fraction of each sample was removed and put through a 10-mL syringe mounted on a silica Sep-Pak that was prewashed with 10 mL of LC mobile phase. A 5-mL volume of LC mobile phase was then passed through the Sep-Pak and collected with the samples application effluent. The collected effluents were taken to dryness under a stream of nitrogen, redissolved in 1 mL of LC mobile phase, and analyzed by HPLC. Standards were prepared as for procedure 2.

Quantitation of Chlorimuron Ethyl in Fortified Samples. As detailed in the above procedure sections, recovery studies were performed in the 0.01–0.10 ppm fortification range. A chlorimuron ethyl standard in either methylene chloride (method 1) or in ethyl acetate(methods 2 and 3) was used for fortifications and preparation of standards. Proper aliquots of this solution were added to soybeans, wheat grain, corn, potato, and turnip controls with Pipetman precision pipettors (100–1000 μL). Proper amounts for standards were pipetted into 13 × 100 mm culture tubes, evaporated to dryness under a stream of nitrogen (nitrogen evaporator), and redissolved in HPLC mobile phase. A standard curve was formed by alternating the injection of standards and samples and using linear regression analysis of the standards to generate the calibration line. The degree of certainty for the line's correlation coefficient was 99% (*r* test), or additional standards were run to assure best "curve fit". Following the calculation of μg/mL the ppm is calculated by

$$\text{chlorimuron ethyl (ppm)} = \frac{\mu\text{g/mL} \times \text{final vol}}{\text{sample wt (g)}}$$

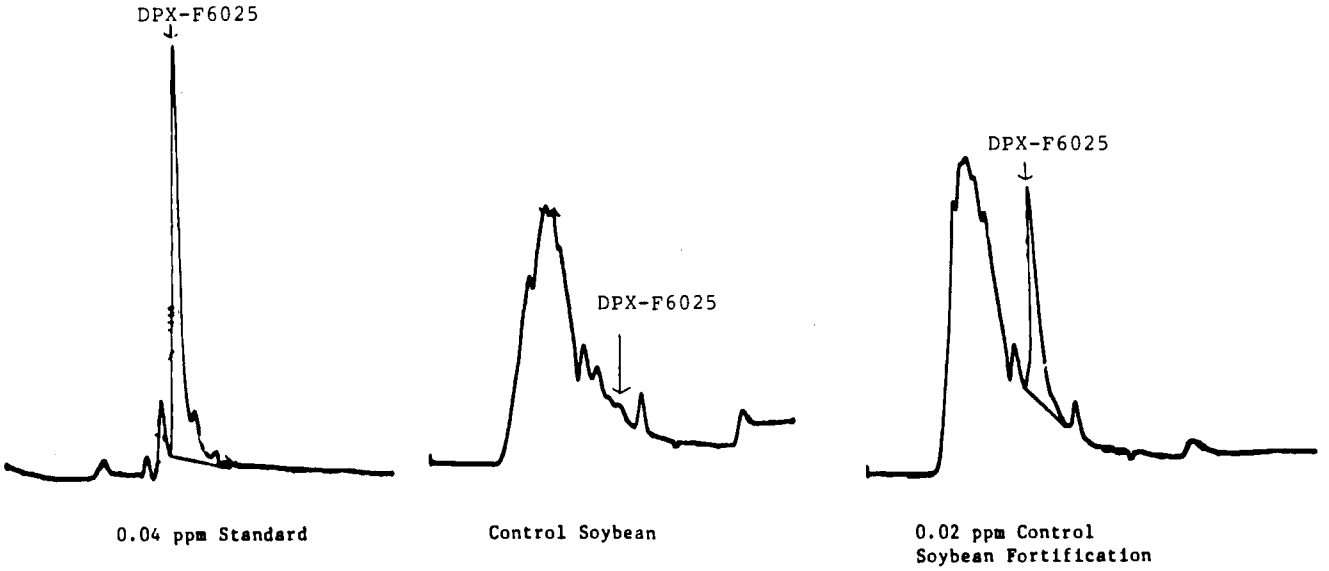

Figure 2. Representative chromatograms from soybean fortified with chlorimuron ethyl (analyzed by method 1).

Table I. Chlorimuron Ethyl Recoveries from Soybeans (Method 1)

fortifcn, ppm	no.	% rec	ISD, %
control	20	nd	
0.01	15	95	5
0.02	15	90	10
0.04	10	91	5
	av	93	±7

RESULTS AND DISCUSSION

Cleanup of soybean extracts was necessary to prevent continuous off-scale response of the detector at required sensitivity settings. Other matrix methods were developed on the basis of the soybean analyses. Specific points concerning each matrix and method are given below.

Method 1. This method has been successfully demonstrated on a large number of soybean samples from various locations. Average recoveries for the soybean samples fortified from the detection level of 0.01 ppm to 0.04 ppm were 93 ± 7% (Table I).

Representative chromatograms of a treated soybean extract and a fortified sample are shown in Figure 2. These chromatograms demonstrate the selectivity and sensitivity of the method and illustrate the fact that no interference was noted in the control samples and a concentration of 0.01 ppm gave more than adequate peak heights.

Method 2. This method was used to analyze nine varieties of soybeans from 10 locations in the midwestern and eastern United States where ground applications of chlorimuron ethyl had been made. Recovery data from methods verification and sample analyses are shown in Table II. Recoveries averaged 92 ± 8.1% for fortifications ranging from 0.01 to 0.10 ppm.

Soybean samples fortified at 0.1 ppm and kept under freezer storage have given complete recovery of residues when checked at seven intervals over 2 years and analyzed by method 2. This demonstrates that chlorimuron ethyl is stable for 2 years under freezer storage conditions for soybean samples.

Wheat grain samples from four locations were also analyzed for chlorimuron ethyl with the data from fortified analyses (0.01–0.1 ppm) given in Table II (average 96 ± 4.9%), and similar methods for verification of recovery data for corn kernels are also presented in Table II (average 97 ± 8.9%).

Table II. Percent Recoveries of Chlorimuron Ethyl Fortifications from Soybeans, Wheat Grain, and Corn Kernels (Method 2)

fortifcn, ppm	% recovery		
	soybean	wheat	corn
0.10	94	93	97
0.05	89	98	98
0.025	92	90	98
0.02	89		
0.01	92	97	90
av, %	92	96	97
std dev	±8.1	±4.9	±8.9
no.	24	13	7

Since the normal-phase HPLC system is sensitive to contamination, sample cleanup is extremely important. These studies have shown that the LiChroprep silica column performs well for the cleanup of soybeans, wheat, and corn kernel extracts. However, when contaminated, the HPLC system should be conditioned with a solution of 40% propanol–40% methanol–20% acetic acid plus 1 mL of H_2O/L of solvent as described in the Experimental Section.

Before the LiChroprep column is calibrated, it should be conditioned with a soybean extract (processed according to method 2). This will provide a consistent elution profile for chlorimuron ethyl. The doublet silica Sep-Pak was not necessary for good cleanup, but it did allow the use of the LiChroprep column for large numbers of samples since compounds that could contaminate the LiChroprep column were retained on the Sep-Pak.

Method 3. Turnip and potato extracts gave no recovery of added fortifications when applied directly to the LiChroprep column. The chlorimuron ethyl would not elute from the column even with methanol. Therefore, the turnip and potato samples were cleaned up by manipulating the chlorimuron ethyl into and out of aqueous solution with pH adjustments. This is possible because of the acid–base ionization characteristics of chlorimuron ethyl. While this cleanup was not as good as the LiChroprep column in that it allowed only 15–20 samples through the HPLC system before reconditioning was required, this detrimental effect would have been worse if Sep-Paks were not used. In the worst-case situation, the HPLC reconditioning would have been required after every five turnip samples if the samples were not passed through a silica Sep-Pak in the LC mobile phase. The silica Sep-

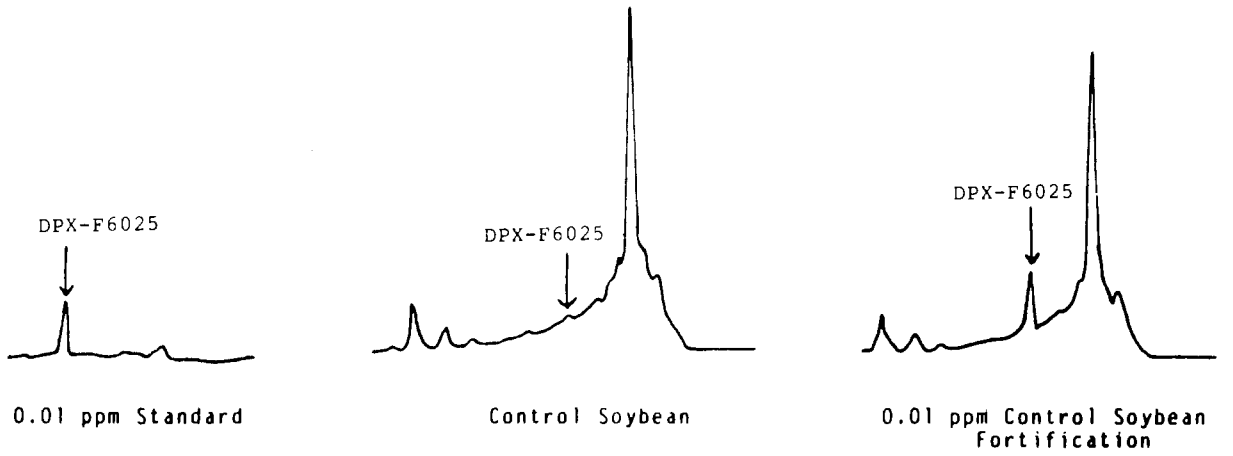

Figure 3. Representative chromatograms from soybeans fortified with chlorimuron ethyl (analyzed by method 2). Wheat grain and corn kernel chromatograms are similar.

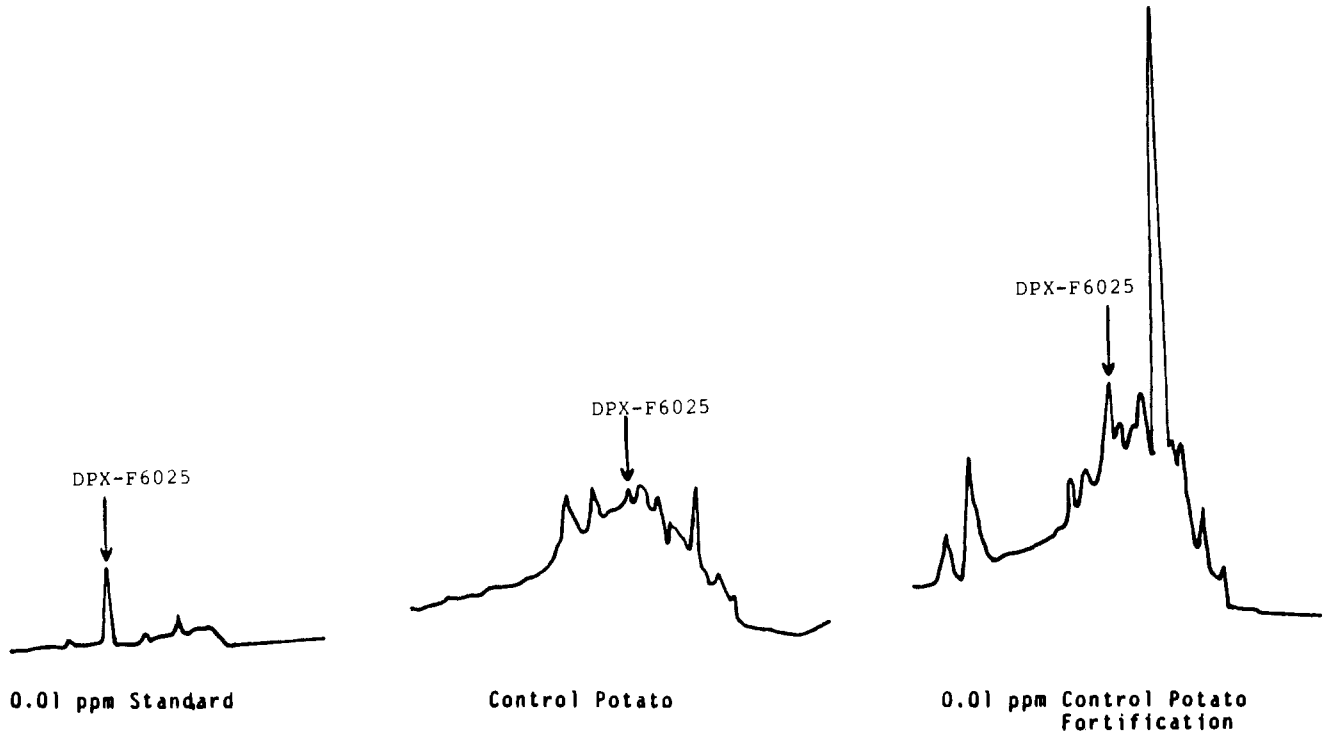

Figure 4. Representative chromatograms from potatoes fortified with chlorimuron ethyl (analyzed by method 3). Turnip chromatograms are similar.

Table III. Percent Recoveries of Chlorimuron Ethyl Fortifications from Turnips and Potatoes

fortificn, ppm	% recovery	
	turnips	potatoes
0.10	110	88
0.05	88	75
0.025		100
0.02	100	
	90	
0.01	100	100
0.01		100
0.01		80
av, %	98	91
std dev	±8.1	±11
no.	5	6

Pak did allow good HPLC sample through-put of potatoes; however, it did not eliminate an interference that eluted from the HPLC column with the same retention time as chlorimuron ethyl. Therefore, the C-18 Sep-Pak was required to reduce the potato interference, although it was not as effective in eliminating the HPLC detrimental effects as the silica Sep-Pak. Recovery data for turnips and potatoes from methods verification and sample analyses (two locations each) are presented in Table III, and representative chromatograms for methods 1–3 are given in Figures 2–4.

ACKNOWLEDGMENT

E. I. du Pont de Nemours and Co. and the authors gratefully acknowledge the expert technical assistance of Don Kalmbacher, Barbara House, and Heriberto Arrabal.

Registry No. Chlorimuron ethyl, 90982-32-4.

LITERATURE CITED

Besenfelder, E. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1981**, *4*, 237.
Beyer, W. F. *Anal. Chem.* **1972**, *44*, 1312.
Bond, W.; Roberts, H. A. *Bull Environ. Contam. Toxicol.* **1976**, *16*, 431.
Brazelton, W. E., Jr.; Ashline, H. C.; Bransome, E. D., Jr. *Anal. Lett.* **1975**, *8*, 301.
Braselton, W. E., Jr.; Bransome, E. D., Jr.; Ashline, H. C.; Stewart, J. T.; Honigberg, I. L. *Anal. Chem.* **1976**, *48*, 1386.

Braselton, W. E., Jr.; Bransome, E. D., Jr.; Huff, T. A. *Diabetes* **1977**, *26*, 50.
Buttler, B.; Hormann, W. D. *J. Agric. Food Chem.* **1981**, *29*, 257.
Hartvig, P.; Fagerlund, C.; Gyllenhall, O. *J. Chromatogr.* **1980**, *181*, 17.
Harzer, K. *J. Chromatogr.* **1980**, *183*, 115.
Hsiao, A. I.; Smith, A. E. *Weed Res.* **1983**, *23*, 231.
Huck, H. *J. Chromatogr.* **1978**, *146*, 533.
Kajinuma, H.; Ichikawa, K.; Akanuma, Y.; Kosaka, K.; Kuzuya, N. *Tonyobyo (Tokyo)* **1982**, *25*, 869.
Kelley, M. M; Zahnow, E. W.; Petersen, W. C.; Toy, S. T. *J. Agric. Food Chem.* **1985**, *33*, 962.
Kimura, M.; Kobayashi, K.; Hata, M.; Takashima, S.; Ino, J.; Matsuoka, A.; Kitamura, H.; Kimura, Y. *Hyogo Ika Daiqaku Igakkai Zasshi* **1980**, *5*, 49.
Kleber, J. W.; Galloway, J. A.; Rodda, B. E. *J. Pharm. Sci.* **1977**, *66*, 635.
Midha, K. K.; McGilveray, I. J.; Charette, C. *J. Pharm. Sci.* **1976**, *65*, 576.
Molins, D.; Wong, C. K.; Cohen, D. M.; Munnelly, K. P. *J. Pharm. Sci.* **1975**, *64*, 123.
Prescott, T. F.; Redman, D. R. *J. Pharm. Pharmacol.* **1972**, *24*, 713.
Raghow, G.; Meyer, M. C. *J. Pharm. Sci.* **1981**, *70*, 1166.
Reinauer, H.; Lindner, G.; Oldendoerp, J. *Fresenius Z. Anal. Chem.* **1980**, *301*, 110.
Robertson, D. L.; Butterfield, A. G.; Kolasinski, H.; Lovering, E. G.; Matsui, F. F. *J. Pharm. Sci.* **1979**, *68*, 577.
Sabih, K.; Sabih, K. *J. Pharm. Sci.* **1970**, *59*, 782.
Simmons, D. L.; Ranz, R. J.; Picotte, P. *J. Chromatogr.* **1972**, *71*, 421.
Slates, R. V. *J. Agric. Food Chem.* **1983**, *31*, 113.
Sved, S.; McGilveray, I. J.; Beaudoin, N. *J. Pharm. Sci.* **1976**, *65*, 1356.
Tsugi, K.; Binns, R. B. *J. Chromatogr.* **1982**, *253*, 227.
Uihlein, M.; Sistovaris, N. *J. Chromatogr.* **1982**, *227*, 93.
Waahlin-Boll, E.; Melander, A. *J. Chromatogr.* **1979**, *164*, 541.
Walters, S. M. *J. Chromatogr.* **1983**, *259*, 227.
Walters, S. M.; Gilvydis, D. M. *LC, Liquid Chromatogr. HPLC Mag.* **1983**, *1*, 302.
Weber, D. J. *J. Pharm. Sci.* **1976**, *65*, 1502.
Zahnow, E. W. *J. Agric. Food Chem.* **1982**, *30*, 854.
Zahnow, E. W. *J. Agric. Food Chem.* **1985a**, *33*, 479.
Zahnow, E. W. *J. Agric. Food Chem.* **1985b**, *33*, 1206.
Zahnow, E. W. *LC, Liq. Chromatogr. HPLC Mag.* **1986**, *4*.

Received for review June 5, 1986. Accepted April 17, 1987.

Determination of *cis*- and *trans*-Aconitic Acids in Plant Materials by Chromatography on Anion-Exchange Resins

John F. Thompson,* Anna-Maria E. Muenster, Timothy S. Artlip,[1] and James T. Madison

A method was devised for the measurement of *cis*- and *trans*-aconitates in plants. This method involves the separation of *cis*- and *trans*-aconitates by elution from a column of a strong anion-exchange resin in the bicarbonate form with an ammonium bicarbonate solution under mild conditions of temperature and pH. *cis*-Aconitate and *trans*-aconitate elute at positions different from those of all the other common naturally occurring organic acids. Quantitative measurement of the aconitates utilizes the formation of color from a mixture of pyridine and acetic anhydride. The chromatographic procedure was shown to avoid the isomerization of *cis*- and *trans*-aconitates. The method was applied to leaf extracts of wheat (*Triticum aestivum* L.) seedlings and gave greater than 98% recovery of added aconitate (5 μmol). Two lots of commercial *cis*-aconitate were contaminated with about 7.0% *trans*-aconitate whereas two batches of commercial *trans*-aconitic acid were over 99% pure.

Many plants can accumulate *trans*-aconitate (TA) to relatively high levels (e.g., up to 6% of the dry weight) (Burau and Stout, 1965; Stout et al., 1967), and this accumulation may be one cause of grass tetany (Mayland and Grunes, 1979). The magnitude of the accumulation can be affected by growing temperature (Stout et al., 1967), root zone temperature (Patterson et al., 1972), and mineral nutrition (Clark, 1968; Barta, 1973). We were interested in understanding the mechanism for aconitate accumulation in plants and required a method for measuring *cis*-aconitate (CA) and TA in plants. A search of the literature revealed that the isomerization of aconitate is facile (Krebs and Eggleston, 1944; Ambler and Roberts, 1948), that most organic acid methods subjected samples to conditions that would cause some isomerization, and that most analysts were more interested in obtaining values for total aconitate rather than accurate values for the individual isomers (Roberts and Ambler, 1947; Poe and Barrentine, 1968; Clark, 1972; Nelson and Rinne, 1977). Molloy (1969) developed a paper chromatographic method that minimizes isomerization. Burau's polarographic method (1969) avoids isomerization conditions except in the last step. He concluded that isomerization was not a serious problem by invoking the results of Ambler and Roberts (1948), whose method of measuring CA and TA is imprecise. Burau (1969) found that his polarographic method caused less isomerization than the silica gel chromatographic method of DeKock and Morrison (1958) and of Coic et al. (1961).

We have devised a procedure for separating CA and TA by salt elution from the bicarbonate form of a strongly basic anion-exchange resin under conditions that avoid isomerization (<0.5%). The separated isomers were quantitated by a colorimetric method.

MATERIALS

CA, TA, *cis*-aconitic acid anhydride (CAA), and *trans*-aconitic acid anhydride (TAA) were purchased from Sigma

U.S. Plant, Soil and Nutrition Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Tower Road, Ithaca, New York 14853.
[1]Present address: Department of Biochemistry, Texas A&M University, College Station, TX 77841.