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Study Title

DETERMINATION OF RESIDUES OF DPX-Y6202, DPX-Y6202 ACID, AND DPX-Y6202 ACID CONJUGATES IN SOYBEANS AND SOYBEAN FRACTIONS

Data Requirement

U.S. EPA Pesticide Assessment Guidelines
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Performing Laboratory

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Laboratory Project ID

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

Company E. I. du Pont de Nemours and Company, Inc.

Company Agent Tony E. Catka (Typed Name) Date 8/13/87

Registration Specialist (Title) Tony E. Catka (Signature)

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GOOD LABORATORY PRACTICE STATEMENT

The GLP requirements specified in 40 CFR Part 160 are not applicable to residue data chemistry requirements at the time of submission.

This study was conducted in the spirit of good laboratory practices.

Study Director L. W. Hershberger Date 1-14-87

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DETERMINATION OF RESIDUES OF DPX-Y6202, DPX-6202 ACID, AND DPX-Y6202 ACID CONJUGATES IN SOYBEANS AND SOYBEAN FRACTIONS

ABSTRACT

A method has been developed to determine DPX-Y6202, DPX-Y6202 Acid and DPX-Y6202 Acid conjugate residues in soybeans and soybean fractions after the conversion of the DPX-Y6202 Acid conjugates to DPX-Y6202 Acid. They are extracted from each soybean or soybean fraction sample (except oils) with a mixture of acetone, water, and glacial acetic acid. After the acetone is evaporated, the remaining aqueous phase is adjusted to pH 5.0 with base.

Soybean oil fractions are dissolved in hexane and the residues extracted into acetonitrile. After the acetonitrile is evaporated, the residue is redissolved in pH 5.0 buffer.

The DPX-Y6202 Acid conjugate for all samples is converted to DPX-Y6202 Acid by incubating with a mixture of ß-glucosidase and cellulase enzymes. The DPX-Y6202 and DPX-Y6202 Acid are then extracted from the aqueous solution into chloroform and the chloroform evaporated. Each sample is then cleaned-up on a medium-pressure LC system which also separates the DPX-Y6202 from

the DPX-Y6202 Acid. The DPX-Y6202 Acid is then derivatized to the methyl ester (ME-DPX-Y6202) with Methyl-8® reagent.

Both the DPX-Y6202 and ME-DPX-Y6202 are quantitated by normal-phase multidimensional HPLC with spectrophotometric detection at 335 nm. Recoveries for 28 soybean and fraction samples fortified with DPX-Y6202 averaged 88% with a standard deviation of 12%. Recoveries for 34 soybean and fraction samples fortified with DPX-Y6202 Acid averaged 88% with a standard deviation of 15%. The detection limit is 0.05 ppm for both compounds for all matrices except soapstock which should be 0.10 ppm.

INTRODUCTION

Ethyl-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy] propanoate (DPX-Y6202) is the active ingredient in Du Pont's new Assure® Herbicide. DPX-Y6202 controls grasses in broadleaf crops such as soybeans. DPX-Y6202 has been shown by M. K. Koeppe (1, 2) and H. Igarashi and co-workers (3, 4) to be metabolized by soybeans to 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy] propanoic acid (DPX-Y6202 Acid). Some of the DPX-Y6202 Acid was shown by these same workers to be conjugated to sugar or cellulose fragments. They showed that the conjugates could be converted to DPX-Y6202 Acid by a mixture of ß-glucosidase and cellulase enzymes. The structures of DPX-Y6202, DPX-Y6202 Acid and the

methyl derivative of DPX-Y6202 Acid (ME-DPX-Y6202) are given in Figure 1.

PROCEDURE

Equipment and Reagents

A Du Pont Model 8800 HPLC (E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware) consisting of a microprocessor controller, Model 870 pump, a column oven, and a data system was used. The column oven was fitted with a Model 7125 Rheodyne injection valve and a Model 7000 Rheodyne switching valve (Rheodyne, Inc., Cotati, California). The pneumatic-actuated switching valve was controlled from the data system through a Rainin Solenoid Interface (Rainin Instruments, Inc., Woburn, Massachusetts). The detector was a Waters Model 481 (Waters Associates, Milford, Massachusetts) spectrophotometer set at 335 nm. The HPLC columns were a Sepralyte® 20H, 4.6 mm x 15 cm, column (Analytichem International, Harbor City, California), #544814, and a Partisil 5 Silica RAC II, 4.6 mm x 10 cm, column (Whatman, Clifton, New Jersey), #L-422-226.

For homogenization and extraction of samples, a Tekmar Tissumizer® (Tekmar Company, Cincinnati, Ohio), Model SDT-1810, with a Model SDT-182 EN shaft and generator was employed. A Vortex-Genie® mixer was used for mixing of samples in centrifuge tubes. An International Equipment Company Model K centrifuge,

fitted with a head to hold six 250 mL centrifuge jars, was used to centrifuge samples. The mixer, centrifuge, and wash bottle were purchased from Fisher Scientific Company, Pittsburgh, Pennsylvania.

A medium-pressure liquid chromatograph (MPLC) (see Figure 2) was constructed from a Rainin Model 653 intermediate-pressure pump (Rainin Instruments), and a Foxy® fraction collector (ISCO, Lincoln, Nebraska). The system also consisted of a Valco 16 position multiport valve (Valco Instruments, Houston, Texas), #ECST 16 PX, and a Valco sample-introduction valve, #GC6PX. The 16 position valve was fitted with 16 2-mL sample loops which were loaded from the sample-introduction valve. The Foxy® fraction collector, through an accessory controller, was used to control the 16-loop Valco valve for automated injection of samples. The medium-pressure column was a Lobar® Size B column (Rainin Instruments), #10608, pre-packed with LiChroprep Si 60 (40-63 mm) packing. A more complete description of the MPLC system is given in Appendix I. Samples can also be cleaned up on a manually operated medium-pressure LC (see Appendix II for a description).

For concentration of samples, a vacuum rotary evaporator with a water bath set at 35°C was used. Pear-shaped flasks (Kontes, Vineland, New Jersey), #K-608700, or 500 mL glass-stoppered erlenmeyer flasks* were used on the rotary

* When using the 500 mL erlenmeyer flasks on a rotary evaporator, the flasks should be taped and the rotary evaporator well shielded to prevent operator injury from a possible implosion.

evaporator. An N-EVAP® evaporator (Organomation Associates, Worcester, Massachusetts) was used to concentrate samples in centrifuge tubes to dryness with nitrogen.

A Millipore all-glass filter apparatus, #XX15 047 00, with a 0.5 µm Teflon® filter, #FHUP 047 00, was used to filter the HPLC solvents (Millipore Corporation, Bedford, Massachusetts). Millipore® Millex®-SR disposable Teflon® filters were used to filter samples before they were loaded on the medium-pressure LC. ACRO®-LC3S or equivalent disposable Teflon® filters (Gelman Sciences, Inc., Ann Arbor, Michigan) were used to filter samples just prior to injection on the HPLC. After extracting, samples were filtered through 75 mL Bond Elut® reservoirs containing 20 µm polyethylene frits. The 75 mL reservoirs and frits were purchased from Analytichem International, Harbor City, California.

Type II ß-glucosidase, #G8625, from almonds and type I cellulase, #C7377, from aspergillus niger (Sigma Chemical, St. Louis, Missouri) were used to hydrolyze the DPX-Y6202 Acid conjugates to DPX-Y6202 Acid. The reference standards of DPX-Y6202, DPX-Y6202 Acid, and ME-DPX-Y6202 were obtained from the Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware. The Methyl 8® reagent was purchased from Pierce Chemical company, Rockford, Illinois. All solvents were distilled-in-glass HPLC grade obtained from Fisher Scientific. All other chemicals were A.C.S. reagent grade obtained from Fisher Scientific.

Isolation

Soybean and Meal Fractions. The entire soybean sample was either ground with a toothmill or else in a blender with dry ice. If dry ice was used, it was allowed to sublime in a freezer before a sub-sample was taken for analysis. Meal fractions were used as received since they were already ground when received.

Either a 10 gram or a 4 gram portion of each soybean grain, hull, meal, flour, or soapstock sample was weighed into a 250 mL glass centrifuge bottle and 75 mL of solution D (see Table 1) added. The sample was then homogenized for 5 min. with the Tissumizer® homogenizer. After homogenizing each sample, the shaft and generator were rinsed with a small portion (<10 mL) of extracting solvent and the rinse was collected in the 250 mL centrifuge bottle for each sample. Any sample caught in the generator was removed using tweezers and also returned to the sample bottle.

After centrifuging the sample at 2000 rpm for 5 min, the liquid was decanted, filtered through a 20 µm frit contained in a 75 mL Bond Elut® reservoir, and collected in a 500 mL erlenmeyer flask. The extraction sequence was repeated, twice more, with an additional 75 mL of solution D each time. These were also filtered and combined with the first extract in the flask.

The sample was then concentrated on a rotary evaporator at 35°C until the organic solvent was removed and only water was

left. If bumping occurred, the flask was removed from the water bath and the temperature of the bath lowered. After the acetone was removed, 50 mL of chloroform* was added and any remaining acetone was evaporated with the chloroform. The samples were then hydrolyzed with the enzymes as described below.

Oil Fractions. A 4 gram portion of oil was weighed into a 250 mL Teflon® centrifuge bottle, 100 mL of hexane added, and the bottle stoppered and shaken for 1 minute to dissolve the oil. 100 mL of 98:2 (acetonitrite:glacial acetic acid) was added and DPX-Y6202 and DPX-Y6202 extracted into the bottom acetonitrite layer by shaking the bottle for 1 minute. The bottom layer was then transferred using a 50 mL glass syringe to a 500 mL glass-stoppered erlenmeyer flask. A second 100 mL extraction with the 98:2 solution was made and the acetonitrite layer combined with the first extract in the erlenmeyer flask.

Each sample was then concentrated on a rotary evaporator at 35°C until the solvent was removed. Then 75 mL distilled water was added and each sample shaken and ultrasonically mixed to dissolve the residue.

Enzyme Hydrolysis

The pH in the 500 mL erlenmeyer flasks for all samples was adjusted to 5.0 ± 0.1 using 1N NaOH and a pH meter. For each

* Chloroform is a suspected weak animal carcinogen. Therefore, gloves should be worn and adequate ventilation provided when handling.

sample, 500 units ß-glucosidase and 200 units cellulase was added and the sample incubated at 37°C overnight (<15 hours) on a shaker bath. The next morning, the samples were removed from the bath and the pH adjusted to 1-2 with 10% HCl using a pH meter.

Each sample was transferred to a 250 mL centrifuge bottle and the erlenmeyer flask rinsed with 100 mL chloroform by shaking and ultrasonically mixing. The chloroform was added to the centrifuge bottle, and the bottle shaken vigorously for 1 minute. After centrifuging for 5 minutes, the chloroform was transferred using a 50 mL glass syringe to a 75 mL Bond Elut® reservoir containing an 8 cm layer of anydrous sodium sulfate and the effluent from the reservoir was collected in a 500 mL pear-shaped flask. The extraction was repeated with 100 mL more chloroform, and the chloroform eluted through the sodium sulfate into the pear-shaped flask. The sodium sulfate was then rinsed with 50 mL of a 1% glacial acetic acid in chloroform solution and the rinse also collected in the pear-shaped flask.

Each sample was evaporated to dryness on the rotary evaporator at 35°C and then transferred to a 13 mL glass-stoppered centrifuge tube with 3 x 3 mL solution A rinses. Each sample was then evaporated to dryness with nitrogen and stored in a refrigerator at <4°C until it was cleaned up on the MPLC.

Medium-Pressure LC Clean Up

Each sample was then dissolved in 4.0 mL of solution E,

(see Table I) centrifuged for 5 minutes and the liquid filtered with a Millex®-SR disposable filter as it was loaded on the MPLC. See Appendix II for manual operation of a medium-pressure LC system. Each sample was used to fill one of the 2.0 mL loops on the 16-loop Valco valve of the MPLC. After each sample had been loaded by the procedure described in Appendix III, they were processed automatically.

Solution E at a flow rate of 5.0 mL/min. was used as the mobile phase for the MPLC. After each sample was injected on the column, the fraction collector was set to discard the column effluent. When the DPX-Y6202 started to elute from the column as determined by a recent calibration (see Appendix IV), the fraction collector switched to collect the DPX-Y6202 in a 50 mL glass-stoppered centrifuge tube (Fraction A). After the DPX-Y6202 had eluted, the fraction collector switched back to the discard position until the DPX-Y6202 Acid started to elute from the column (determined by a recent calibration). The fraction collector then switched to collect the DPX-Y6202 Acid in another 50 mL glass-stoppered centrifuge tube (Fraction B). After the DPX-Y6202 Acid had eluted, the fraction collector switched back to the discard position. The column was then washed for 20 minutes with solution E before the next sample was injected. A typical timing sequence for the MPLC system is given in Table 2.

Fractions (A) and (B) for each sample were evaporated to dryness on the N-EVAP® evaporator. The DPX-Y6202 Acid was then

derivatized to ME-DPX-Y6202 by adding 500 µL of Methyl-8®* reagent to fraction B in the centrifuge tube. After dissolving the residue, the sample was heated in a Pierce Reacti-Therm® heating block at 105°C for 1 hour. After the reaction was complete, each sample was cooled to room temperature and then evaporated to dryness on the N-EVAP® evaporator. Both fractions A and B were stored at <4°C until analyzed by HPLC.

For HPLC analysis, each fraction A was redissolved in 1.0 mL of solution A (see Table 1) for 4 gram samples and 2.5 mL for 10 gram samples, whereas Fraction B was redissolved in 1.0 mL of solution B for 4 gram samples and 2.5 mL for 10 gram samples. Both fractions were filtered through ACRO®-LC3S disposable filters; the A fractions were analyzed for DPX-Y6202 and the B fractions for ME-DPX-Y6202.

High Performance Liquid Chromatography

DPX-Y6202. A Du Pont Model 8800 HPLC fitted with a high-pressure switching valve (time programmed from a Du Pont data system) and a Waters Model 481 UV spectrometer set at 335 nm was used for the analysis. The oven temperature was 40°C and the injection volume was 100 µL. The attenuation of the detector was set at 0.005 AUFS and any further attenuation of samples or standards was on the data system.

* Since the toxicity of Methyl 8 is not well known, it should always be handled in a hood and gloves should be worn.

A diagram of the columns and switching valve arrangement is shown in Figure 3. The first column, C_1, was a Sepralyte® 20H column and the second column, C_2, was a Partisil 5 Silica RAC II column. In valve position I (see Figure 3), the effluent from C_1 went through a 10 µL bypass loop, back to the valve, and to the detector. In the other position, II, the effluent from C_1 went to C_2, back to the valve, and then to the detector.

Table 3 gives a typical timing sequence for analysis of samples for DPX-Y6202. At the time of injection, the valve was in position I (see Figure 2) and C_2 was bypassed. The mobile phase, solution A (see Table 1), was pumped at a flow rate of 1.0 mL/min. When DPX-Y6202 started to elute from C_1 at 4.08 min., the valve was switched to position II. After DPX-Y6202 had eluted from C_1 at 4.33 min., the valve was switched back to position I. While the valve was in position II, the DPX-Y6202 was trapped on C_2 and held there for later elution. The valve switching times at 4.08 and 4.33 min. were set at -0.15 and +0.10 min. around the retention time for DPX-Y6202 on C_1.

After the DPX-Y6202 had been trapped on C_2, the mobile phase was switched to solution B (see Table 1) at 5.0 min. and the flow rate increased to 4.0 mL/min. to clean off C_1. After C_1 had been cleaned off and equilibrated to solution B, the flow was decreased to 1.0 mL/min., and the valve switched at 15.0 min. to position II to elute the DPX-Y6202 from column C_2.

When elution of DPX-Y6202 and other peaks from C_2 was completed at 25.0 min., the valve was switched back to position I. The mobile phase was changed to solution A and the flow rate raised to 3.0 mL/min. to quickly re-equilibrate C_1 to solution A. After 5 min. (30.0 min. total), the flow rate was decreased to 1.0 mL/min. and the next sample or standard injected.

ME-DPX-Y6202. The same HPLC system and approach was used for ME-DPX-Y6202 as was used for DPX-Y6202. For ME-DPX-Y6202, solution B was used as the mobile phase for both columns (see Table 1).

Table 4 gives a timing sequence for analysis of samples for ME-DPX-Y6202.

Standard Preparation

DPX-Y6202. A stock standard of DPX-Y6202 at 100 µg/mL was made by dissolving 10 mg of DPX-Y6202 in 100 mL of acetonitrile. Dilutions at 0.1, 0.2, 0.5, and 1.0 µg/mL in acetonitrile were made from the stock. HPLC standards were made by evaporating to dryness 3.0 mL of these standards and then redissolving them in solution A.

DPX-Y6202 Acid. A stock standard of DPX-Y6202 Acid at 100 µg/mL was made by dissolving 10 mg of DPX-Y6202 Acid in 100 mL of acetonitrile.

ME-DPX-Y6202. A stock standard of ME-DPX-Y6202 at 100 µg/mL was made by dissolving 10 mg in 100 mL of acetonitrite. Dilutions at 0.1, 0.2, 0.5, and 1.0 µg/mL in acetonitrite were made from the stock. HPLC standards were made by evaporating to dryness 3.0 mL of these standards and then redissolving them in solution B.

Fortifying Standards. Fortifying standards containing both DPX-Y6202 and DPX-Y6202 Acid in equal concentrations were made in acetonitrile at 0.2, 0.5, 1.0, and 3.0 µg/mL.

Calculations

DPX-Y6202. The response factor for each standard, R, in mg/(mm-mL) units was calculated by the equation.

$$R = C_S / [(P_S)(A)]$$

C_S was the concentration of the standard in µg/mL units, P_S was the peak height in millimeter and A was the data system attentuation. The average response factor, R_a, for standards injected interspersed with samples was calculated and used for calculation of DPX-Y6202 residues in samples.

The sample concentration, C, in ppm units was calculated using the equation.

$$C = \frac{(P)(V)(R_a)(F)(A)(D)}{W}$$

P was the sample peak height in millimeters, and V was the final sample volume in mL. F was the sample aliquot factor (2) which came from the MPLC clean up, D was the dilution factor for any samples which were diluted for HPLC analysis, and W was the sample weight in grams.

DPX-Y6202 Acid. The concentration of DPX-Y6202 Acid in samples was calculated using the equation:

$$C = \frac{(P)(V)(Ra)(F)(A)(D)}{(W)(1.04)}$$

Where the terms are the same as defined for DPX-Y6202. The factor of 1.04 is the molecular weight ratio of ME-DPX-Y6202 to DPX-Y6202 Acid.

RESULTS

Several changes to the procedure described in revision B have been made in this revision (Revision 3). The procedure has been changed to include analysis of soybean fractions such as flour and oils in the main body of the procedure rather than as an appendix. Because of interference from recent batches of enzymes in the analysis of samples for DPX-Y6202 Acid, the final quantitation has been changed. DPX-Y6202 Acid is quantitated as the methyl derivative (ME-DPX-Y6202) after methylation with Methyl 8® reagent. To solubilize the final extract and reduce

background interferences, the detection limit has also been raised from 0.02 ppm to 0.05 ppm for DPX-Y6202 Acid (except for soapstock). The DPX-Y6202 detection limit has also been raised to 0.05 ppm because of interferences and to compare with the detection limit for DPX-Y6202 Acid. Although we ran a recovery at 0.05 ppm DPX-Y6202 for the soapstock the detection limit should be 0.10 ppm. Because of the high pH of soapstock, DPX-Y6202 can not be detected but is converted to DPX-Y6202 Acid.

The procedure was modified recently to include the enzyme hydrolysis step with cellulase and ß-glucosidase. H. Igarashi and co-workers (3, 4) have shown that in mature soybeans from 10 to 20% of the total ^{14}C residues are present as conjugates. These conjugates when hydrolyzed with cellulase and ß-glucosidase enzymes were converted to DPX-Y6202 Acid. Therefore, the procedure has been modified to include these enzymes using the conditions for the hydrolysis obtained from the work of H. Igarashi and co-workers (3,4).

The extraction of DPX-Y6202 Acid from field treated samples was compared to results obtained by M. K. Koeppe for a ^{14}C-DPX-Y6202 treated sample (2). Both the work by M. K. Koeppe and our comparison did not include analysis for DPX-Y6202 Acid conjugate. The sample used for the comparison was treated on August 14, 1984 at 10 oz ai/A with [quinoxaline-^{14}C]-DPX-Y6202. M. K. Koeppe reported a DPX-Y6202 Acid concentration of 0.07 ppm. We analyzed the same sample six times and found concentrations

ranging from 0.04 to 0.06 ppm with an average of 0.05 ppm. We were, therefore, able to extract 70% of the DPX-Y6202 Acid present. If this is corrected for processing recovery of 88%, the percent recovered is raised to 80%. It can be expected, therefore, that with the addition of the enzyme hydrolysis step, the majority of the residues of DPX-Y6202, DPX-Y6202 Acid, and conjugates can be recovered.

Either 4.0 gram or 10.0 gram samples were used during analysis of samples and to generate recoveries. We recommend that 4.0 gram samples be used to reduce the amount of sample loaded on the Lobar column and thereby to increase its life time. For 4.0 gram samples a volume of 1.0 mL should be used for the final HPLC extract, except for soapstock which should be 2.0 mL.

DPX-Y6202

Recovery data for soybean grain and fraction samples fortified with DPX-Y6202 are given in Table 5. DPX-Y6202 recoveries for 28 soybean and fraction samples averages 88% with a standard deviation of 12%

Fraction A chromatograms of a control soybean sample, the same sample fortified with DPX-Y6202, and a soybean sample from Newark, Delaware treated with DPX-Y6202 are shown in Figures 4, 5, and 6. Fraction A chromatograms of control soybean flour and crude oil samples, the same samples fortified with DPX-Y6202, and

soybean flour and crude oil samples from a processing study where the beans were treated with DPX-Y6202 are shown in Figures 7 to 12.

DPX-Y6202 Acid

Recovery data for soybean seed and fraction samples fortified with DPX-Y6202 Acid are given in Table 6. DPX-Y6202 recoveries for 34 soybean and fraction samples averaged 88% with a standard deviation of 15%

Fraction B chromatograms of a control soybean sample, the same sample fortified with DPX-Y6202 Acid, and a soybean sample from Talleysville, Virginia treated with DPX-Y6202 are shown in Figures 13, 14, and 15. Fraction B chromatograms of control soybean flour, crude oil, and soapstock samples, the same samples fortified with DPX-Y6202 Acid, and soybean flour, crude oil, and soapstock samples from a processing study where the beans were treated with DPX-Y6202 are shown in Figures 16 to 24.

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TABLE 1

Solution Compositions
(Volume in mL)

Solvent	Solution A	Solution B	Solution D	Solution E
Hexane	980	930	---	580
Tetrahydrofuran	---	30	---	---
Acetone	---	---	750	400
Glacial Acetic	20	40	2	20
Water	---	---	250	---

TABLE 2

Timing Sequence For The MPLC

Time Range (min.)	Compound Eluted	Fraction Collected
0 to 17	None	Waste
17 to 20	DPX-Y6202	A
20 to 22	None	Waste
22 to 26	DPX-Y6202 Acid	B
26 to 40	None	Waste

TABLE 3

HPLC Timing For DPX-Y6202 Analysis

Time Range (min.)	Mobile Phase	Flow Rate (mL/min.)	Valve Position	Columns
0.0 to 4.08	Solution A	1.0	I	C_1
4.08 to 4.33	Solution A	1.0	II	$C_1 + C_2$
4.33 to 5.00	Solution A	1.0	I	C_1
5.00 to 15.00	Solution B	4.0	I	C_1
15.00 to 25.00	Solution B	1.0	II	$C_1 + C_2$
25.00 to 30.00	Solution A	3.0	I	C_1

TABLE 4

HPLC Timing For DPX-Y6202 Acid Analysis

Time Range (min.)	Mobile Phase	Flow Rate (mL/min.)	Valve Position	Columns
0.0 to 4.99	Solution B	1.5	I	C_1
4.99 to 5.24	Solution B	1.5	II	$C_1 + C_2$
5.24 to 6.00	Solution B	1.5	I	C_1
6.00 to 17.00	Solution B	4.0	I	C_1
17.00 to 28.00	Solution B	2.0	II	$C_1 + C_2$

TABLE 5

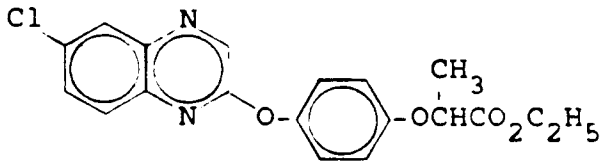
DPX-Y6202 Recoveries

Matrix	Number of Samples	Fortification Range	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)
Soybeans	12	0.05 - 0.30	62 - 108	85	16%
Soybean Flour	6	0.05 - 0.25	80 - 92	88	6%
Soybean Hulls	2	0.05 - 0.25	88 - 96	92	--
Soybean Oil	8	0.05 - 0.25	80 - 100	92	7%
				Total = 88	12%

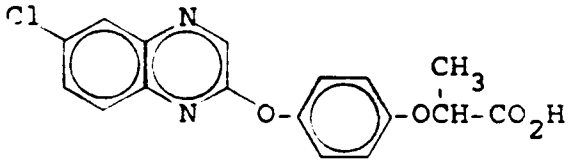
TABLE 6

DPX-Y6202 Acid Recoveries

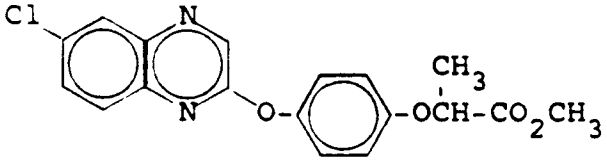
Matrix	Number of Samples	Fortification Range	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)
Soybeans	16	0.05 - 0.25	66 - 110	91	13%
Soybean Flour	6	0.05 - 0.25	84 - 116	102	14%
Soybean Hulls	2	0.05 - 0.25	96 - 112	104	--
Soybean Oil	8	0.05 - 0.25	60 - 100	77	12%
Soybean Soapstock	2	0.05 - 0.25	73 - 103	88	--
				Total = 88%	15%



DPX-Y6202



DPX-Y6202 ACID



ME-DPX-Y6202

FIGURE 1: The structures of DPX-Y6202, DPX-Y6202 Acid and the methyl derivative of DPX-Y6202 Acid (ME-DPX-Y6202).

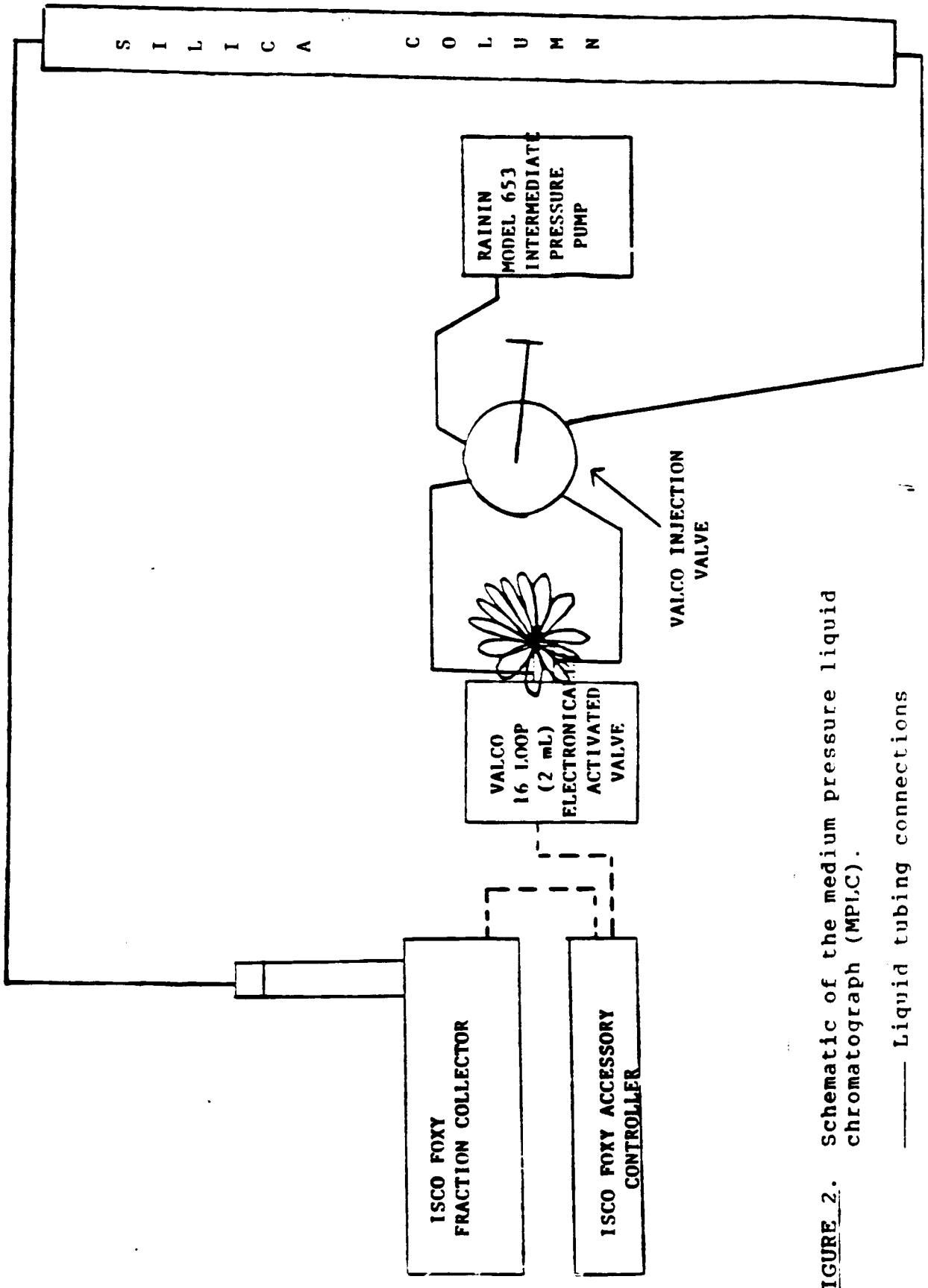


FIGURE 2. Schematic of the medium pressure liquid chromatograph (MPLC).

——— Liquid tubing connections

- - - - - - Electrical connections

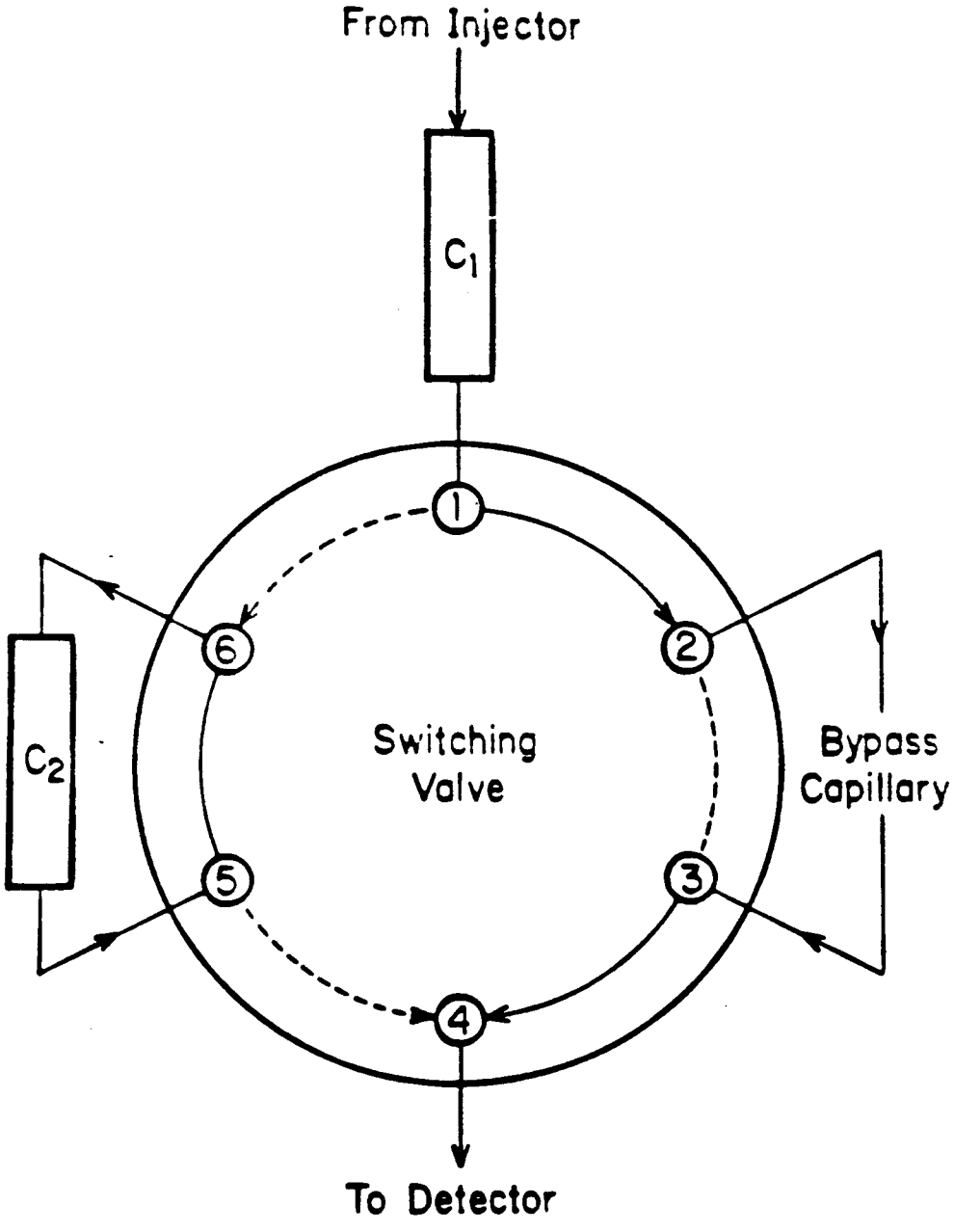


FIGURE 3. Chromatographic column arrangment.

——— Position I
------ Postion II

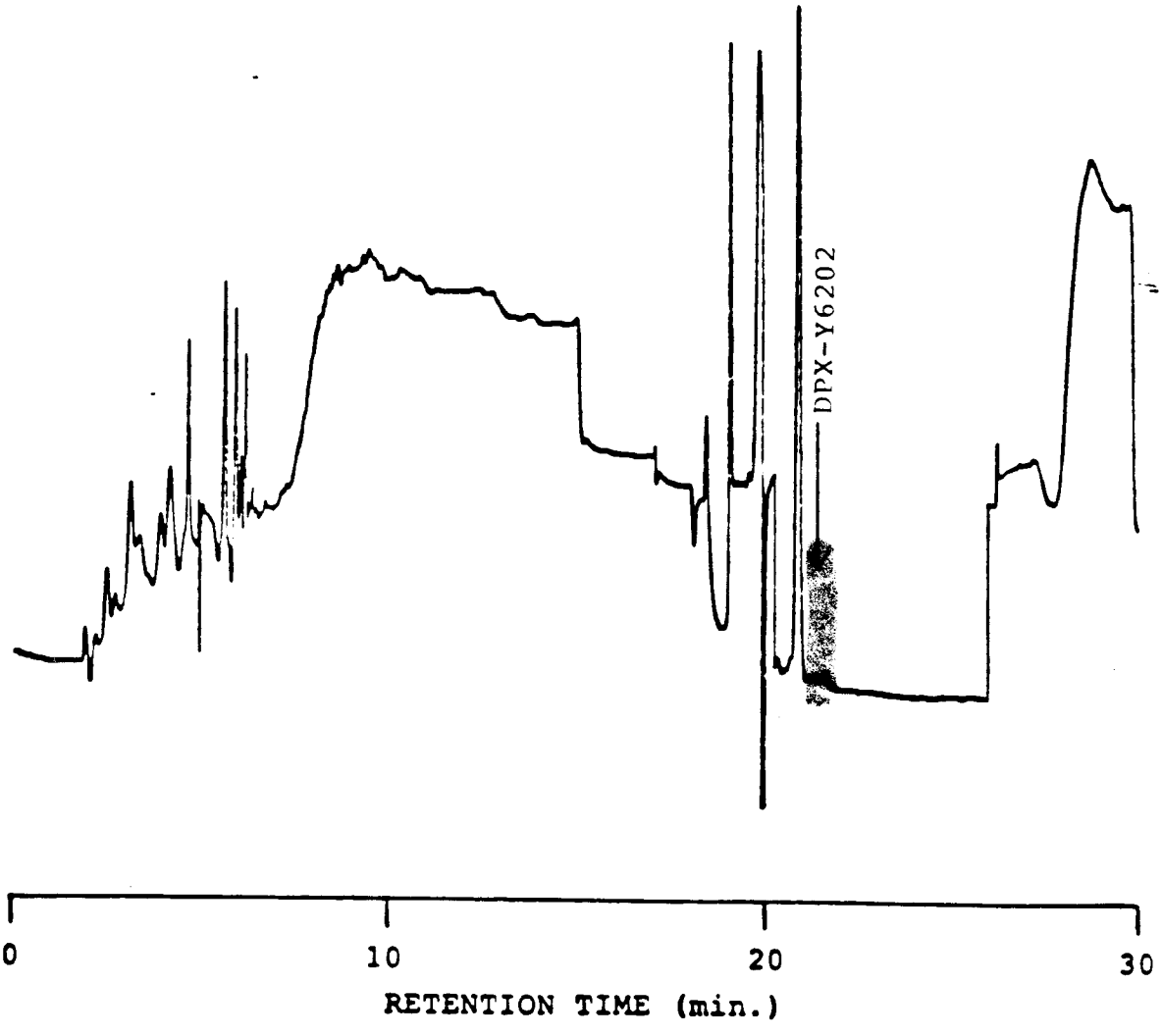


FIGURE 4: Chromatogram of fraction A from a [redacted] sample from Newark, Delaware.

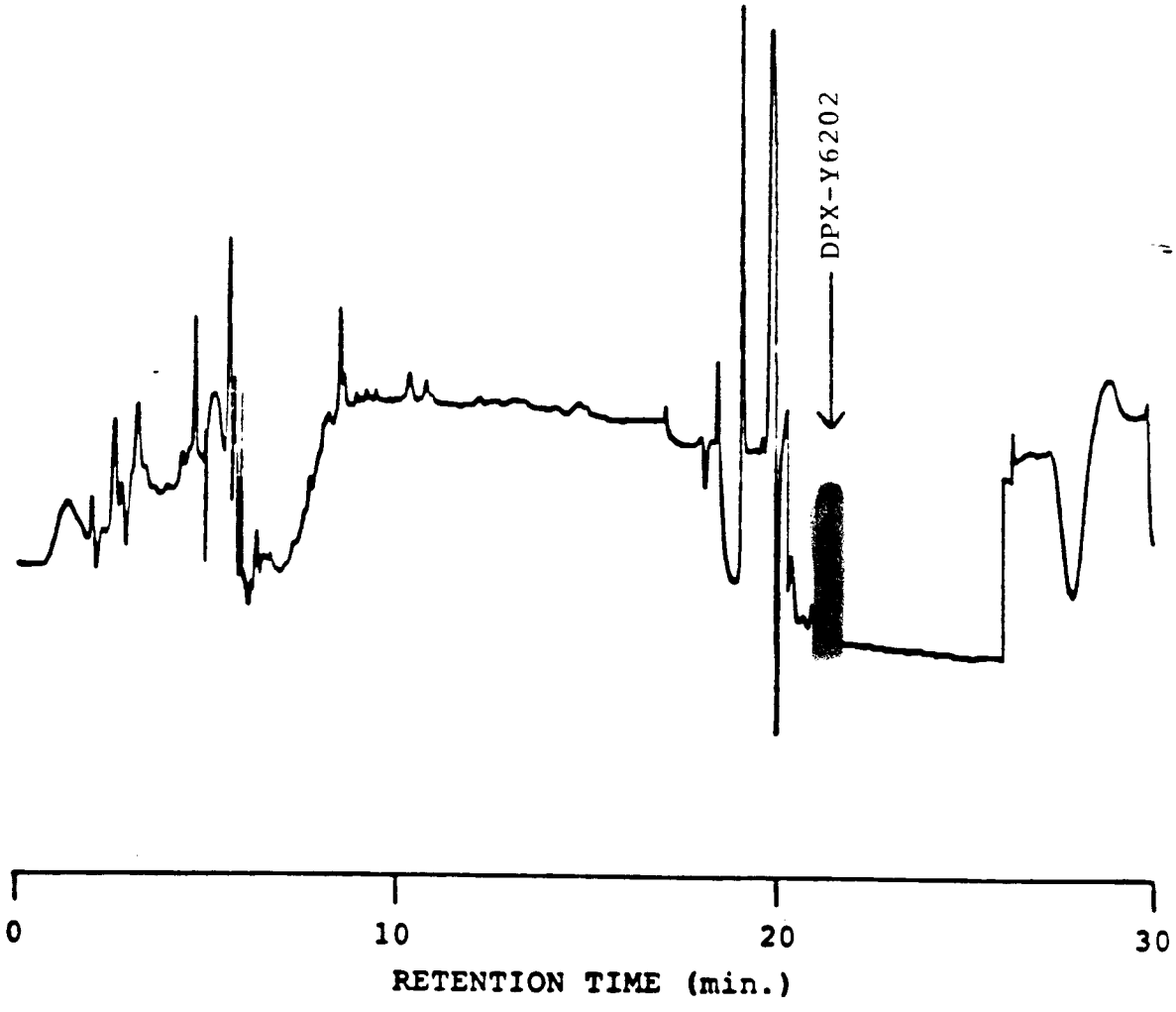


FIGURE 5: Chromatogram of fraction A from the same control soybean sample as in Figure 4 fortified with 0.05 ppm DPX-Y6202 (Recovery = 80%).

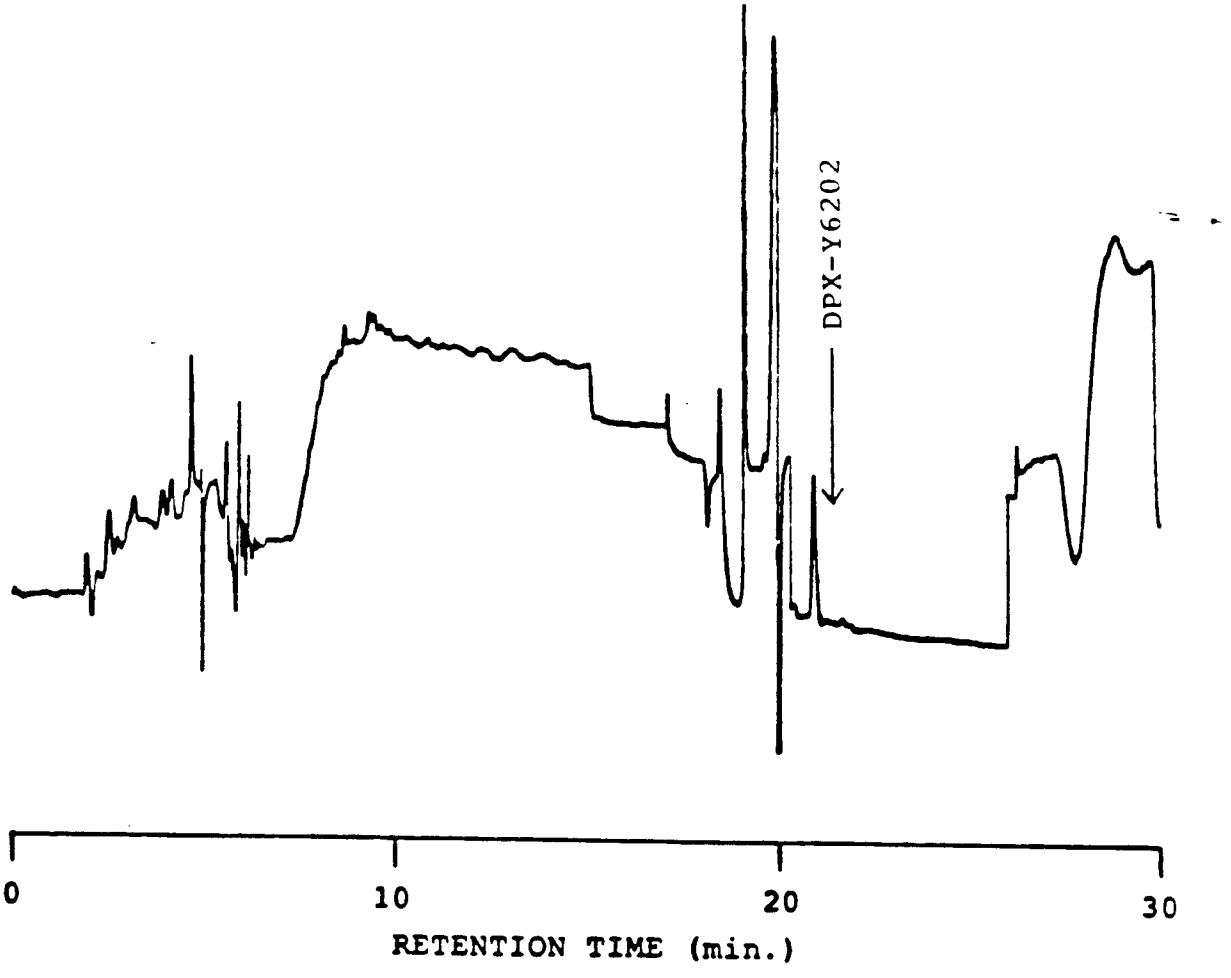


FIGURE 6: Chromatogram of fraction A from a soybean sample from Newark, Delaware treated at 8 ounce active ingredient per acre with DPX-Y6202 and sampled 75 days later.

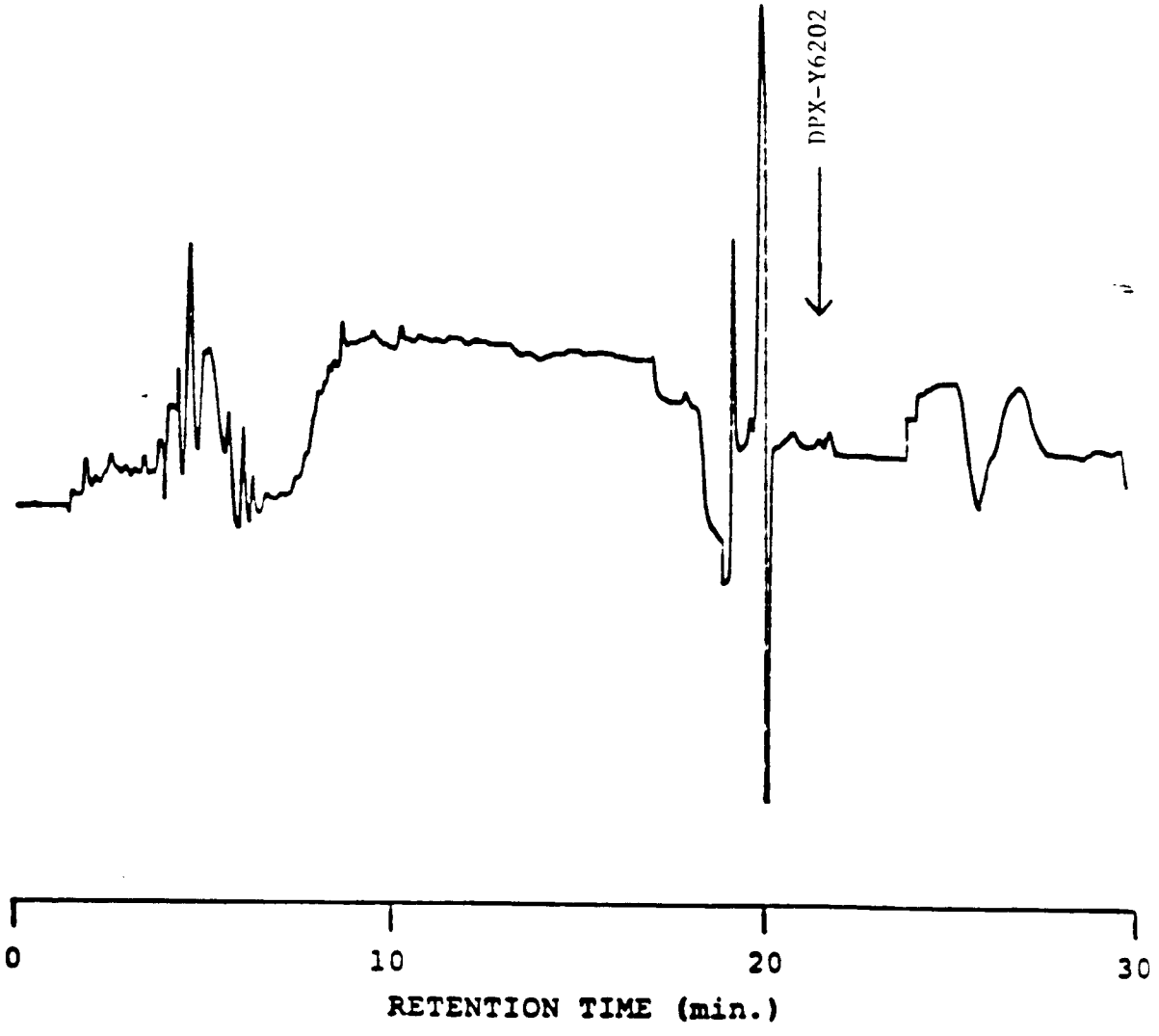


FIGURE 7: Chromatogram of fraction A from a control soybean flour sample from a processing study.

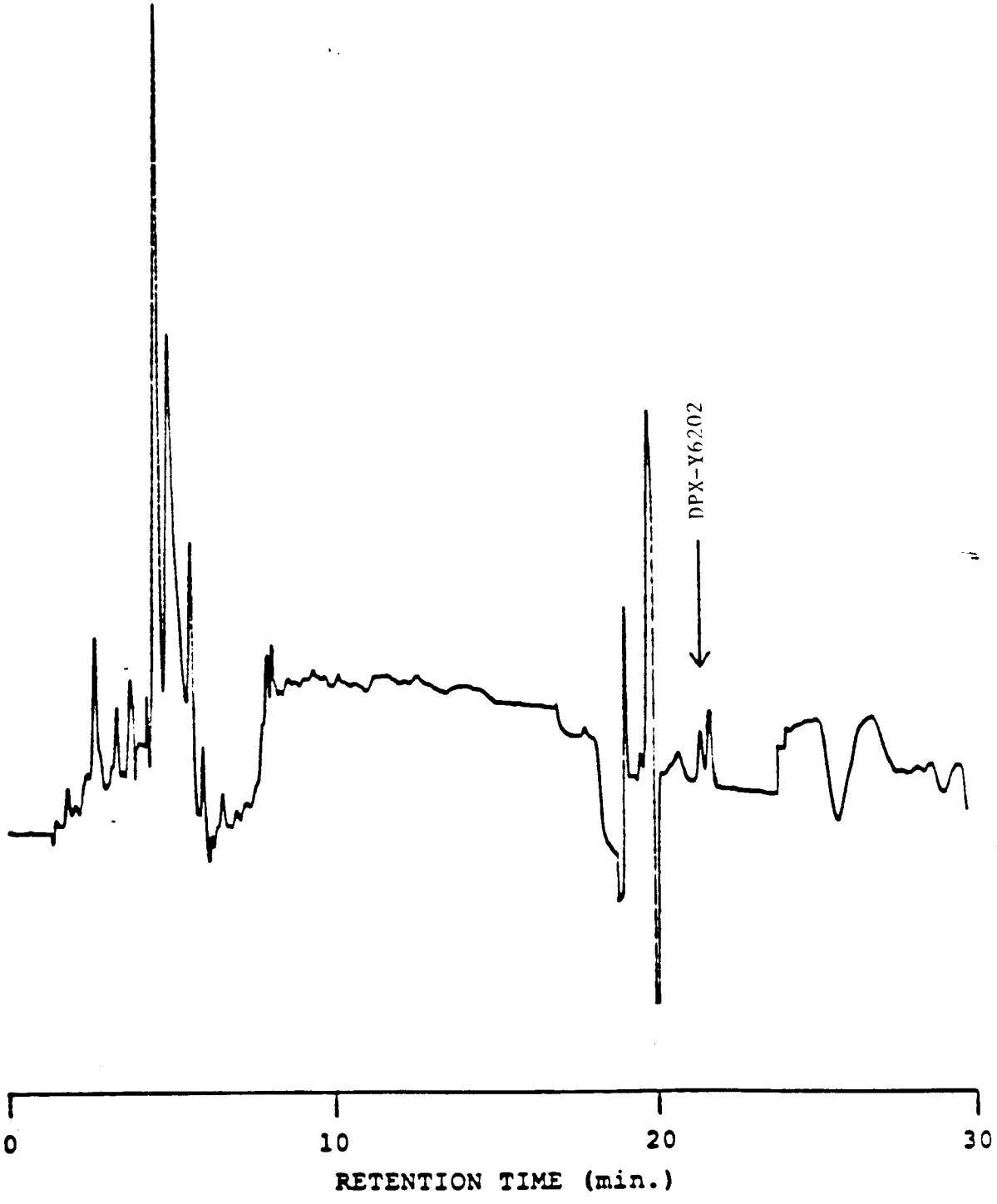


FIGURE 8: Chromatogram of fraction A from the same control soybean flour sample as in Figure 7 fortified with 0.05 ppm DPX-Y6202 (Recovery = 80%).

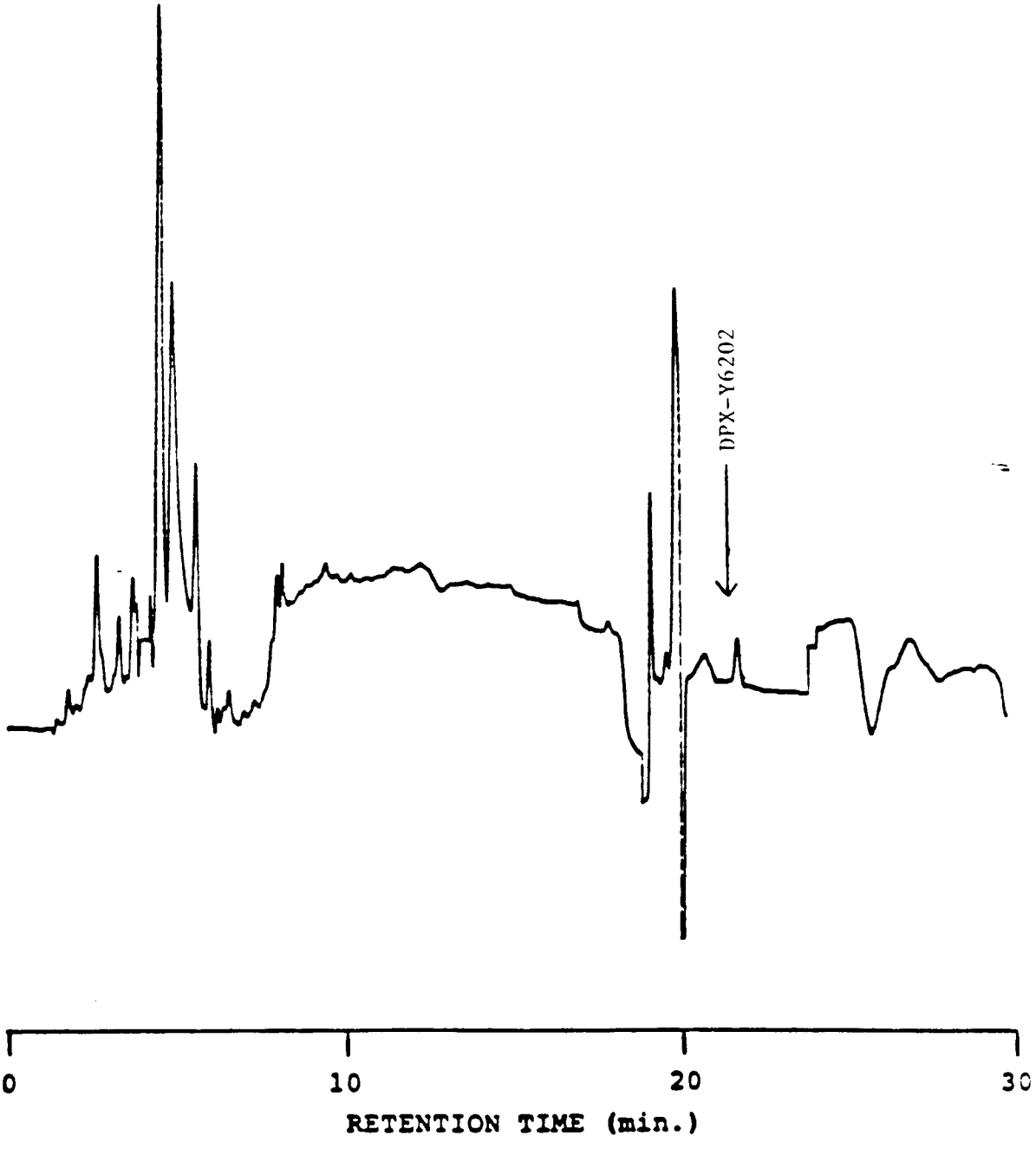


FIGURE 9: Chromatogram of fraction A from a soybean flour sample from a processing study where the soybeans were treated at 8 oz ai/A with DPX-Y6202 and sampled 72 days later.

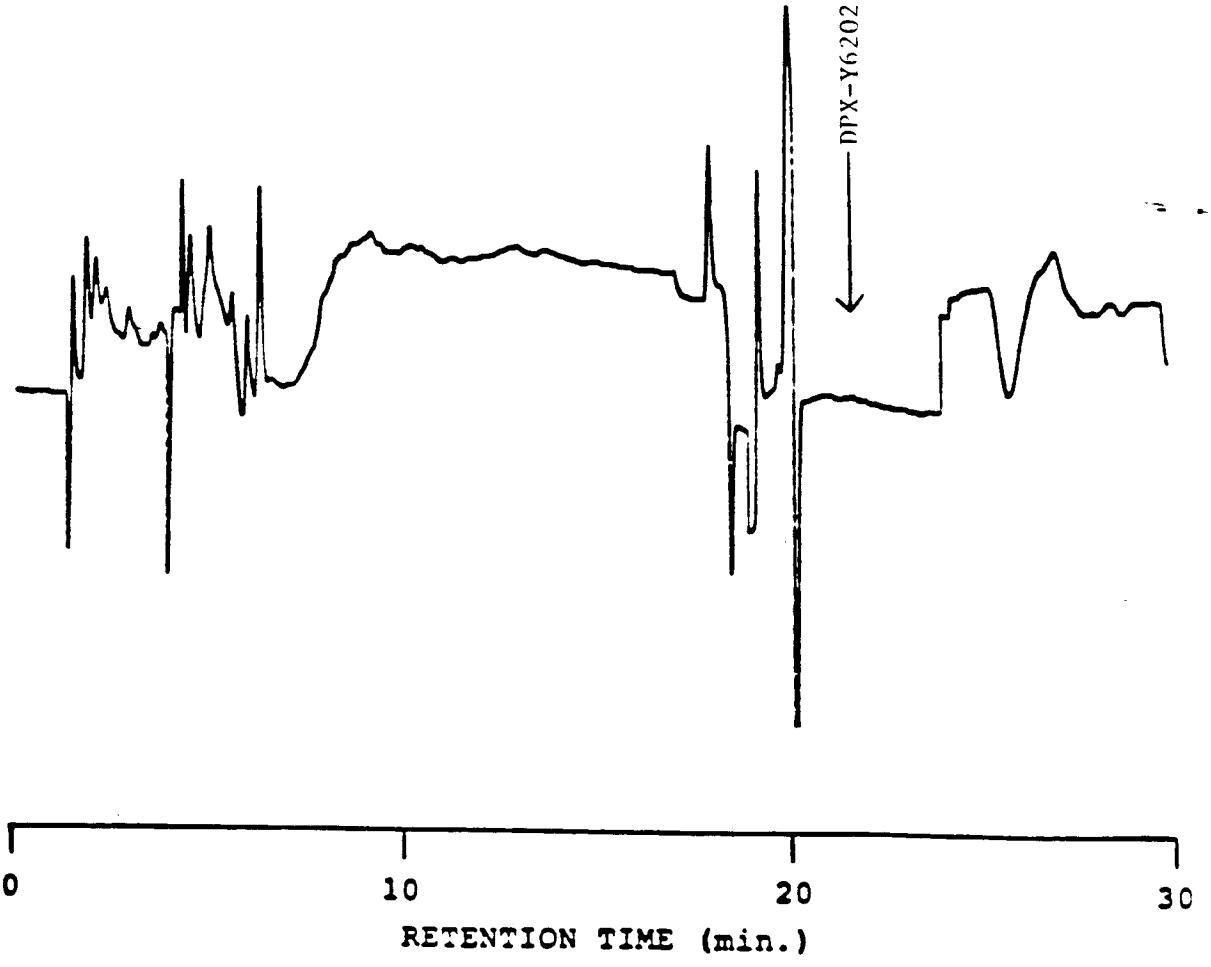


FIGURE 10: Chromatogram of fraction A from a control soybean crude oil sample from a processing study.

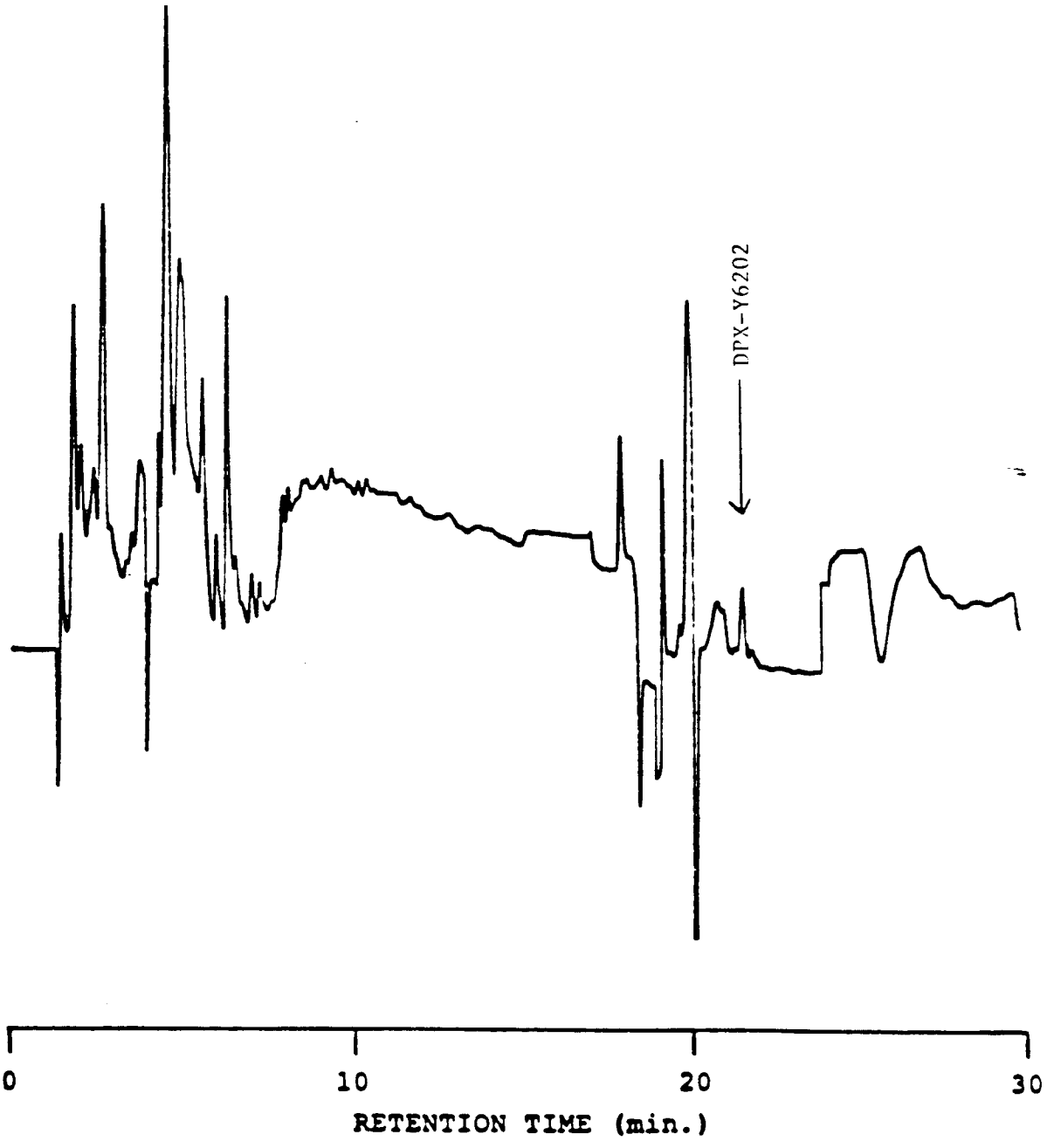


FIGURE 11: Chromatogram of fraction A from the same control soybean crude oil sample as in Figure 10 fortified with 0.05 ppm DPX-Y6202 (Recovery = 100%).

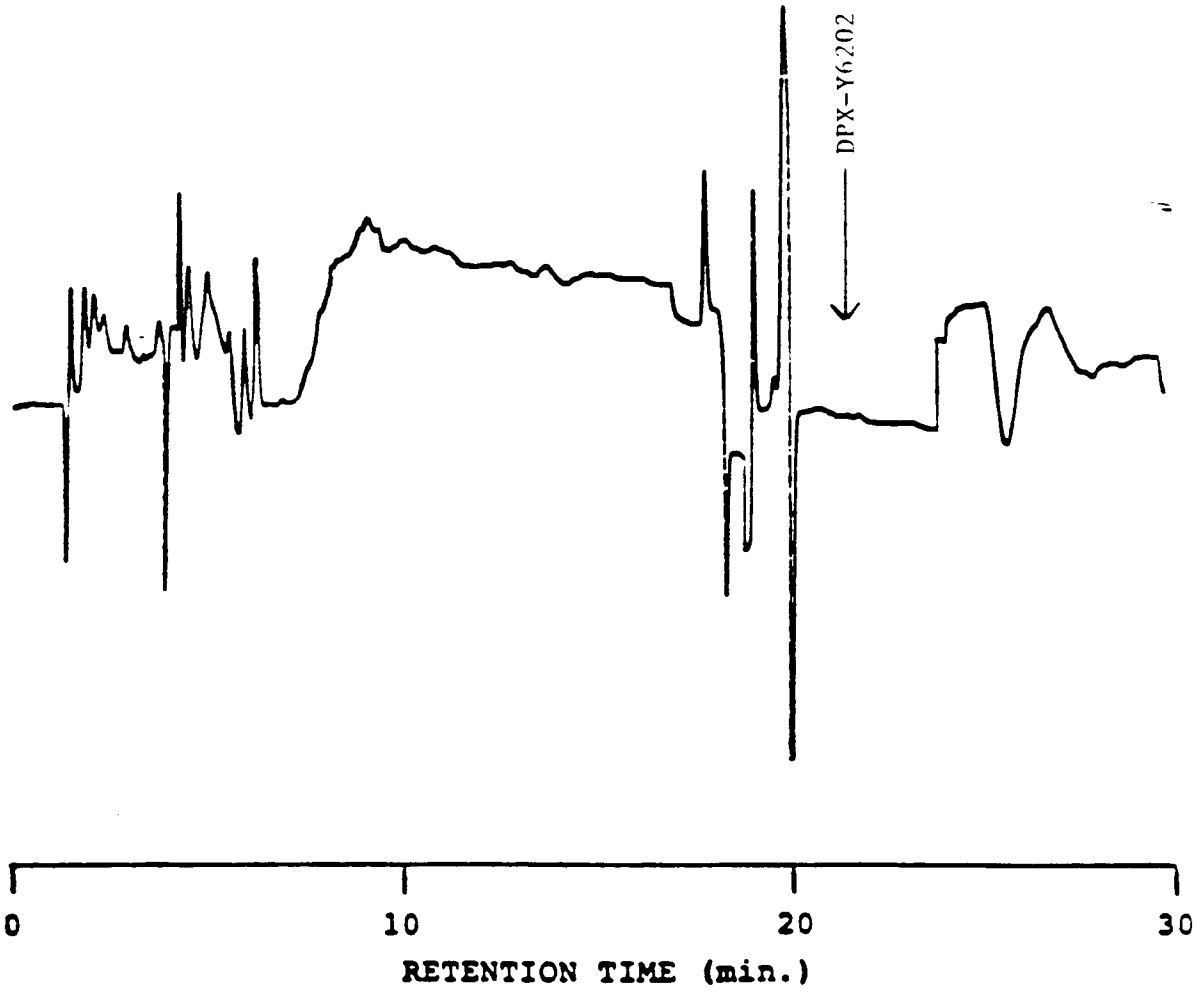


FIGURE 12: Chromatogram of fraction A from a soybean crude oil sample from a processing study where the soybeans were treated at 8 oz ai/A with DPX-Y6202 and sampled 72 days later.

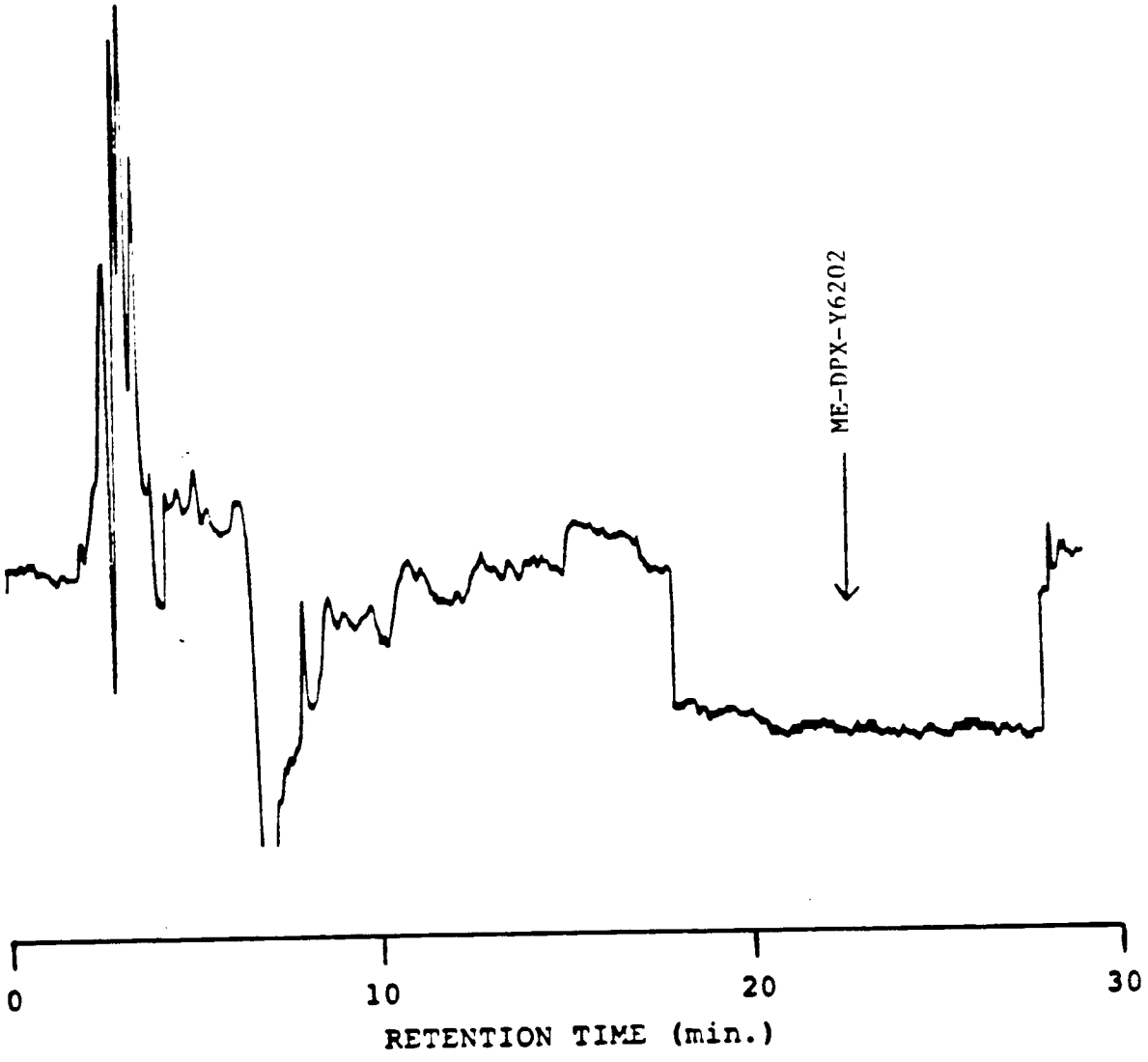


FIGURE 13: Chromatogram of fraction B from a control soybean sample from Talleysville, Virginia.

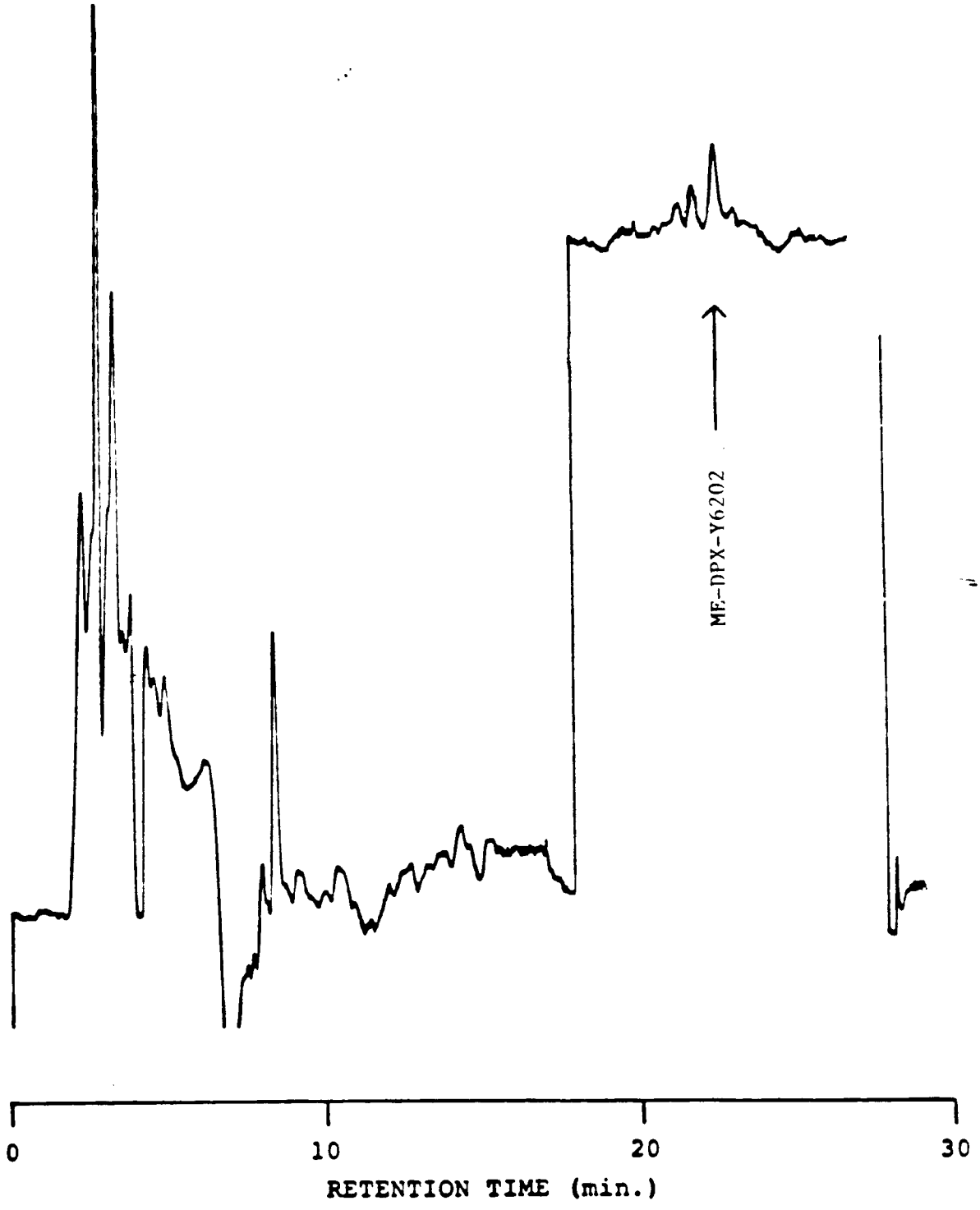


FIGURE 14: Chromatogram of fraction B from the same soybean sample as Figure 13 fortified with 0.05 ppm DPX-Y6202 Acid (Recovery = 88%).

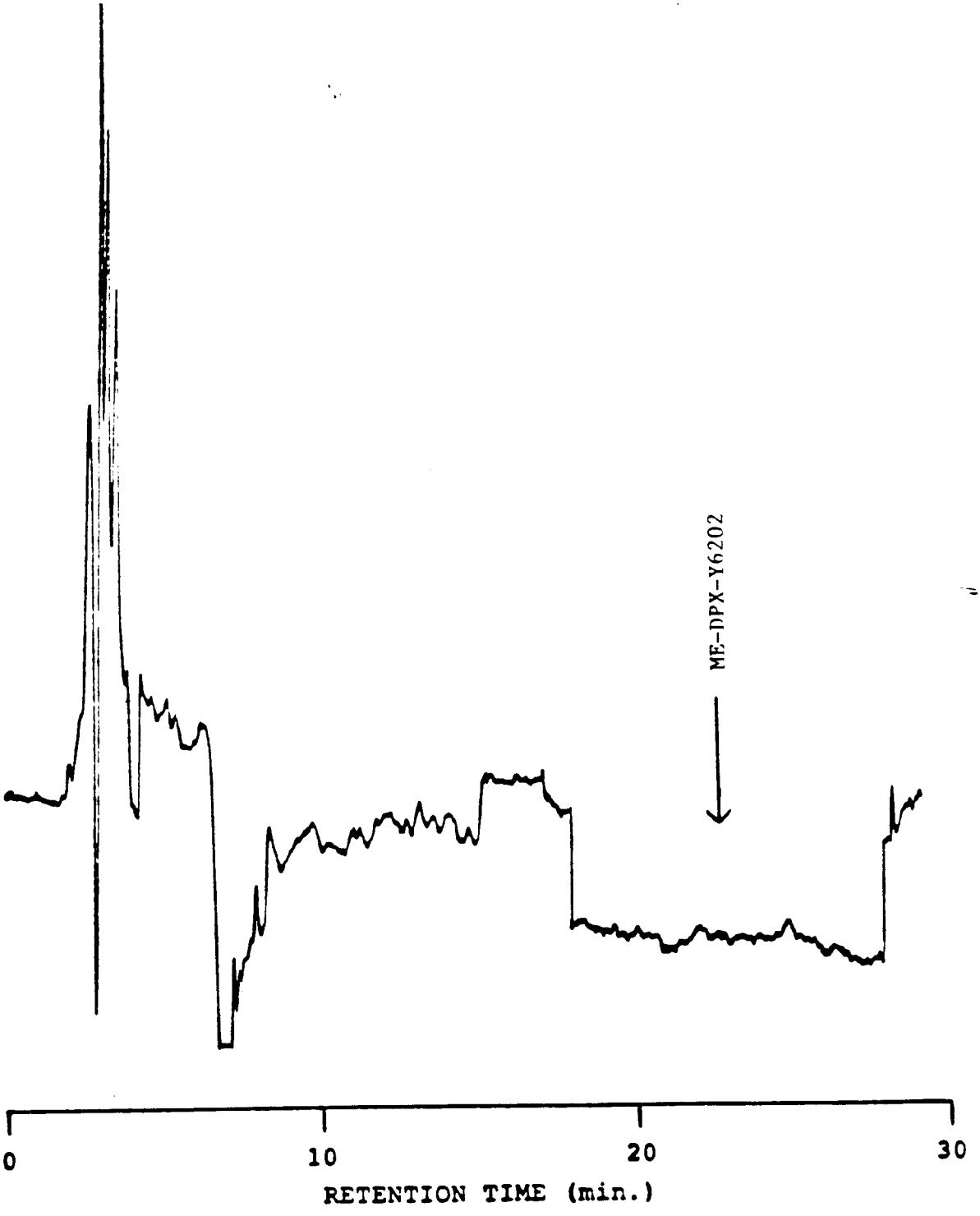


FIGURE 15: Chromatogram of fraction B from a soybean sample from Talleysville, Virginia treated with 8 oz ai/A DPX-Y6202 and sampled 84 days later.

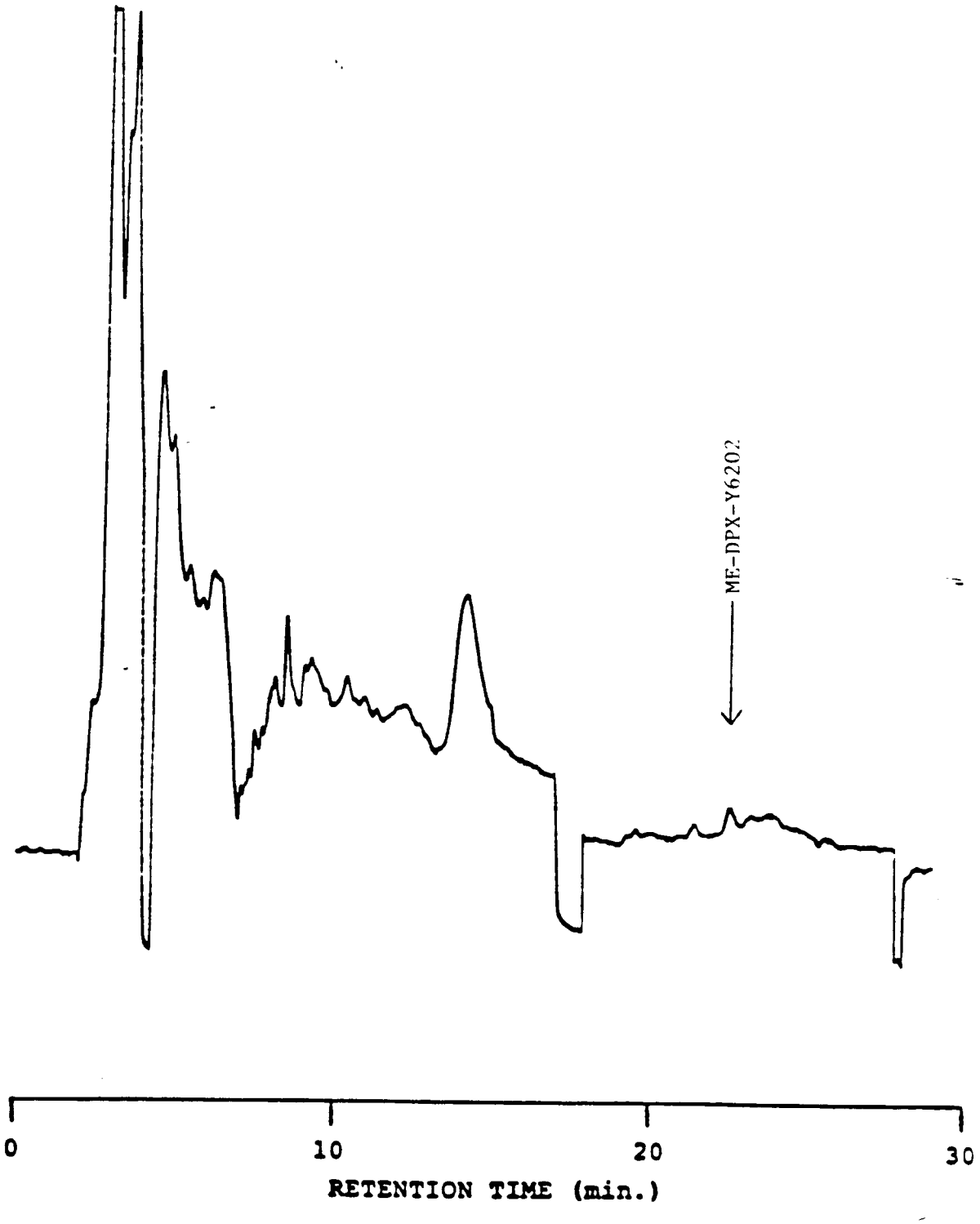


FIGURE 16: Chromatogram of fraction B from a control soybean flour sample from a processing study (DPX-Y6202 Acid <0.05 ppm).

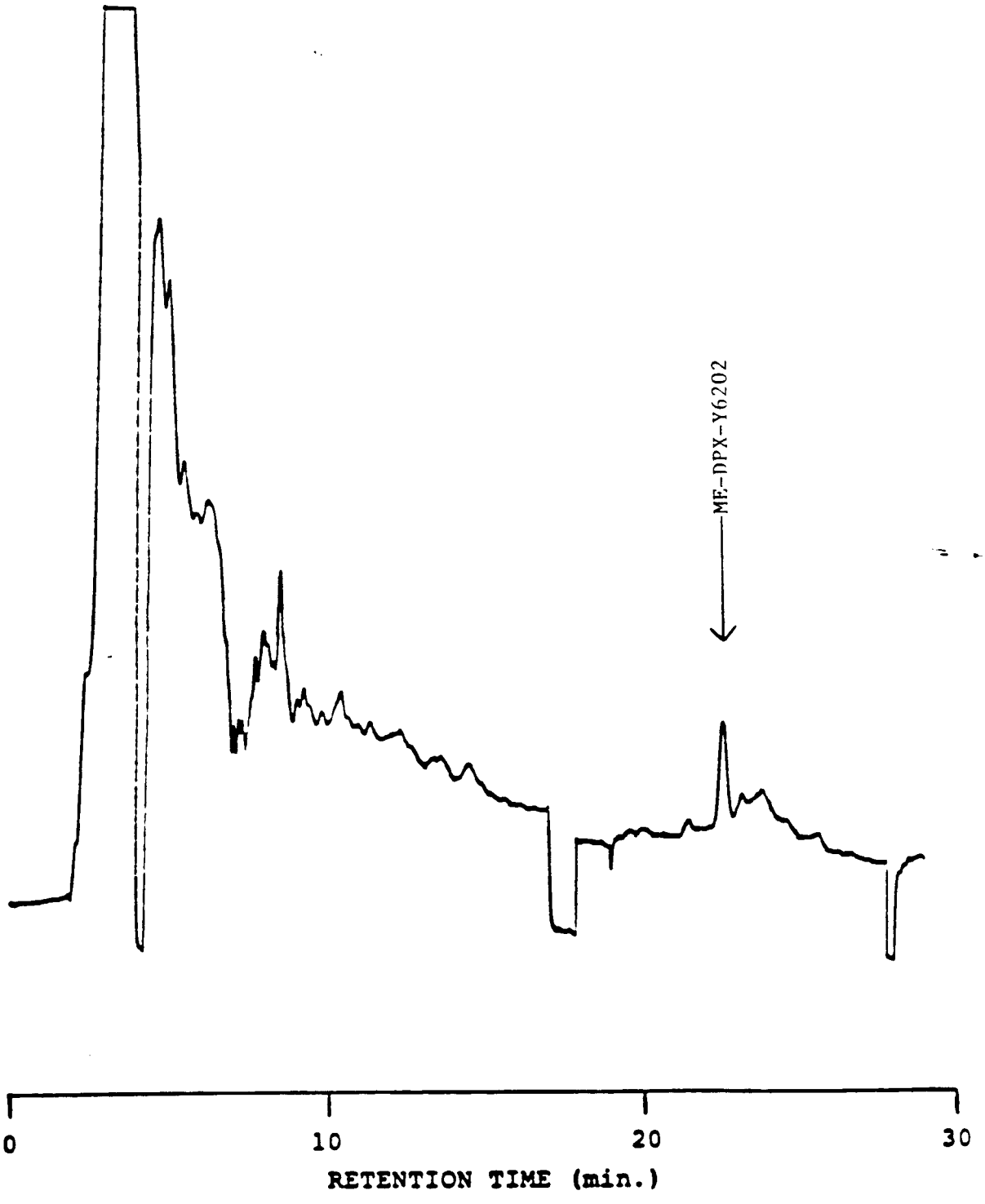


FIGURE 17. Chromatogram of fraction B from the same contact soybean flour sample as in Figure 16 fortified with 0.05 ppm DPX-Y6202 Acid (Recovery = 115%)

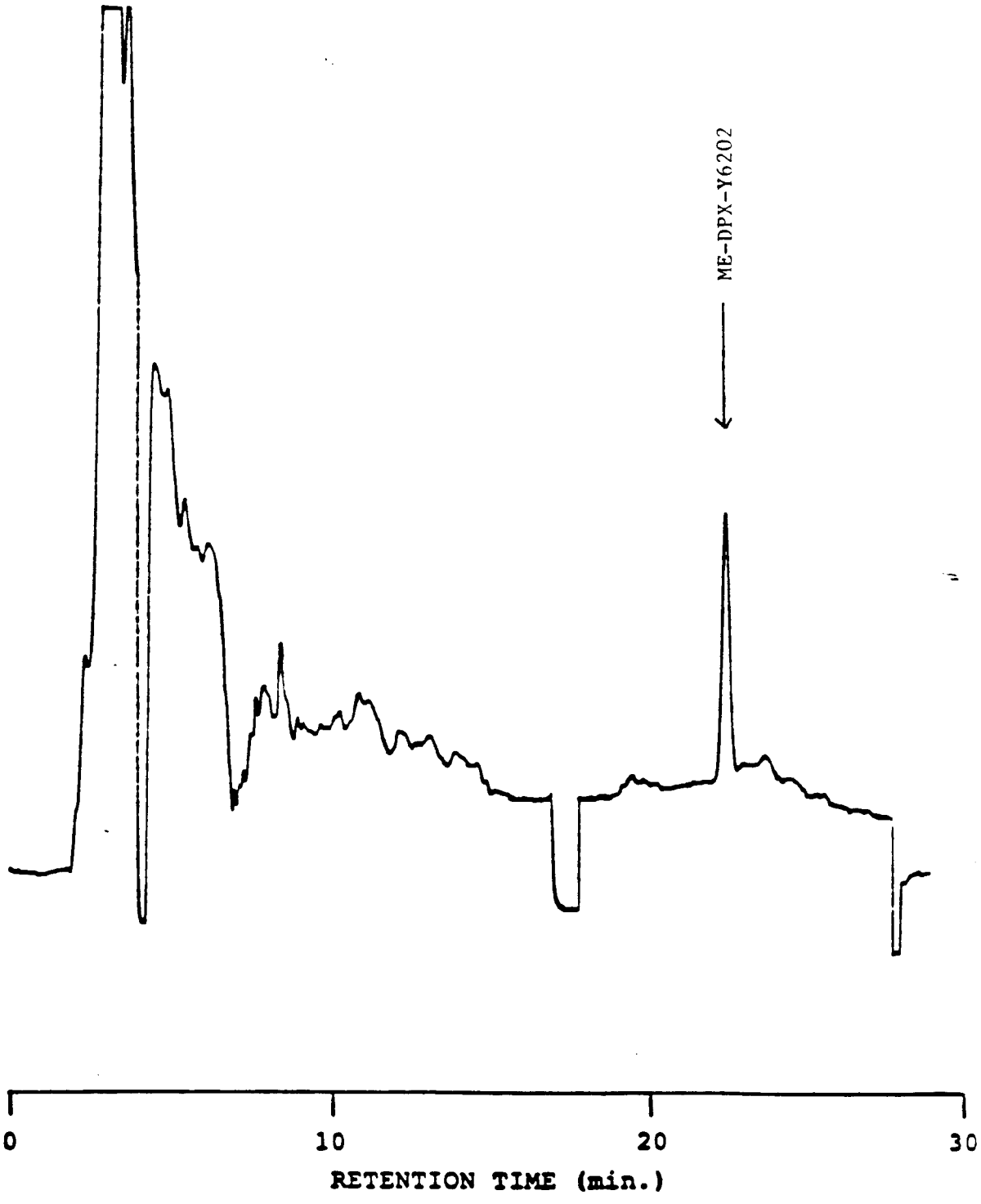


FIGURE 18: Chromatogram of fraction B from a soybean flour sample from a processing study where the soybeans were treated at 8 oz ai/A with DPX-Y6202 and sampled 72 days later (DPX-Y6202 Acid = 0.19 ppm).

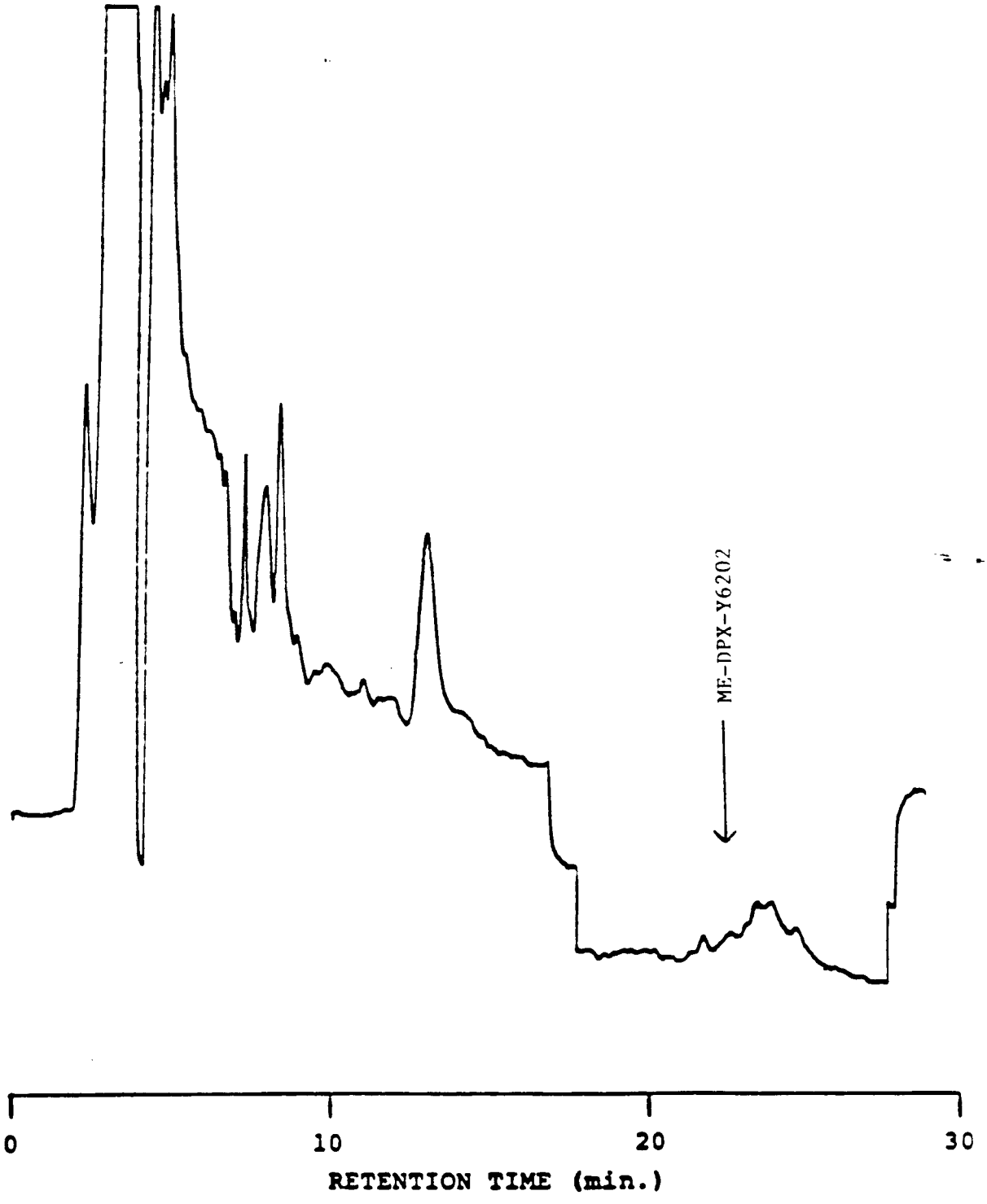


FIGURE 19. Chromatogram of fraction B from a control soybean crude oil sample from a processing study.

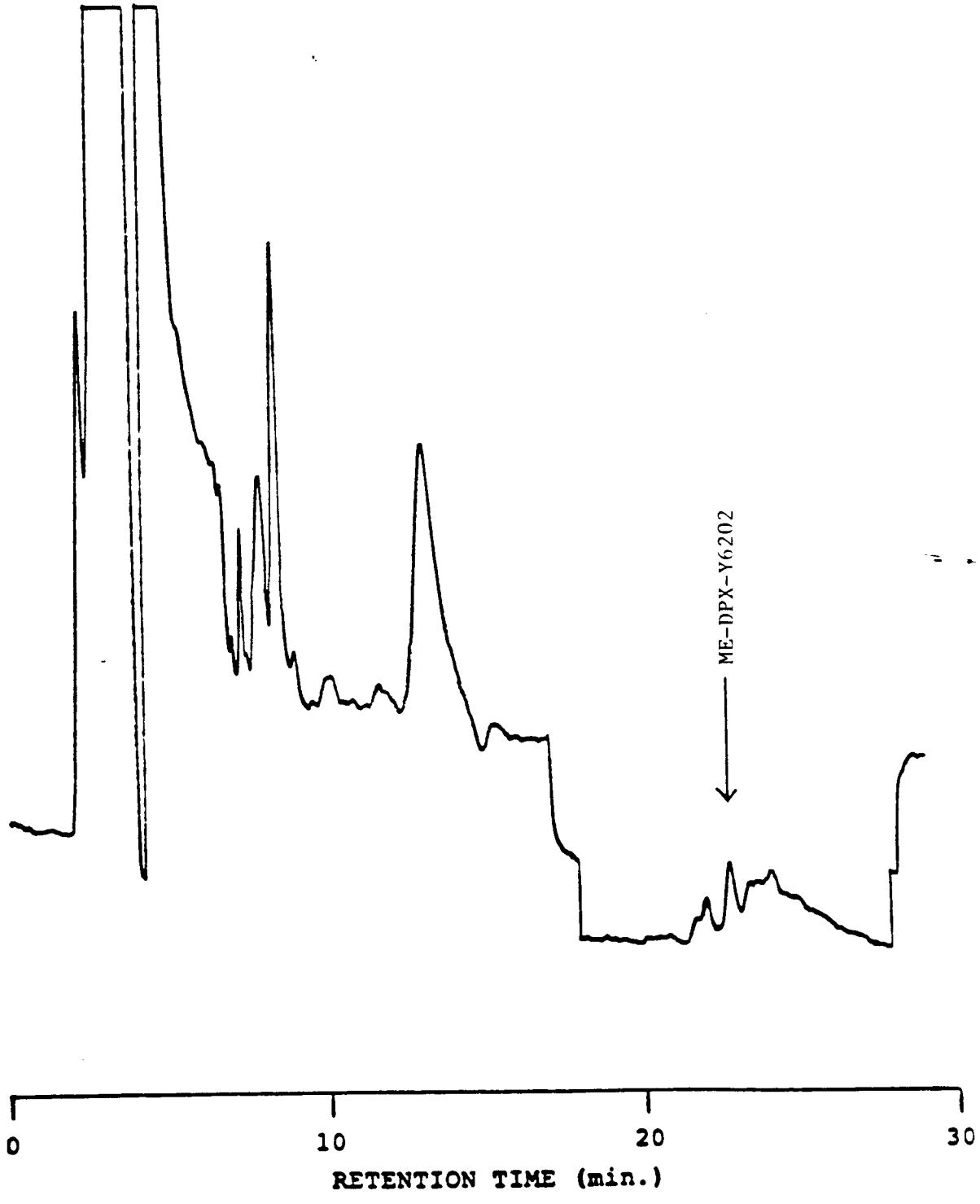


FIGURE 20: Chromatogram of fraction B from the same control soybean crude oil sample as in Figure 19 fortified with 0.05 ppm DPX-Y6202 Acid (Recovery = 80%).

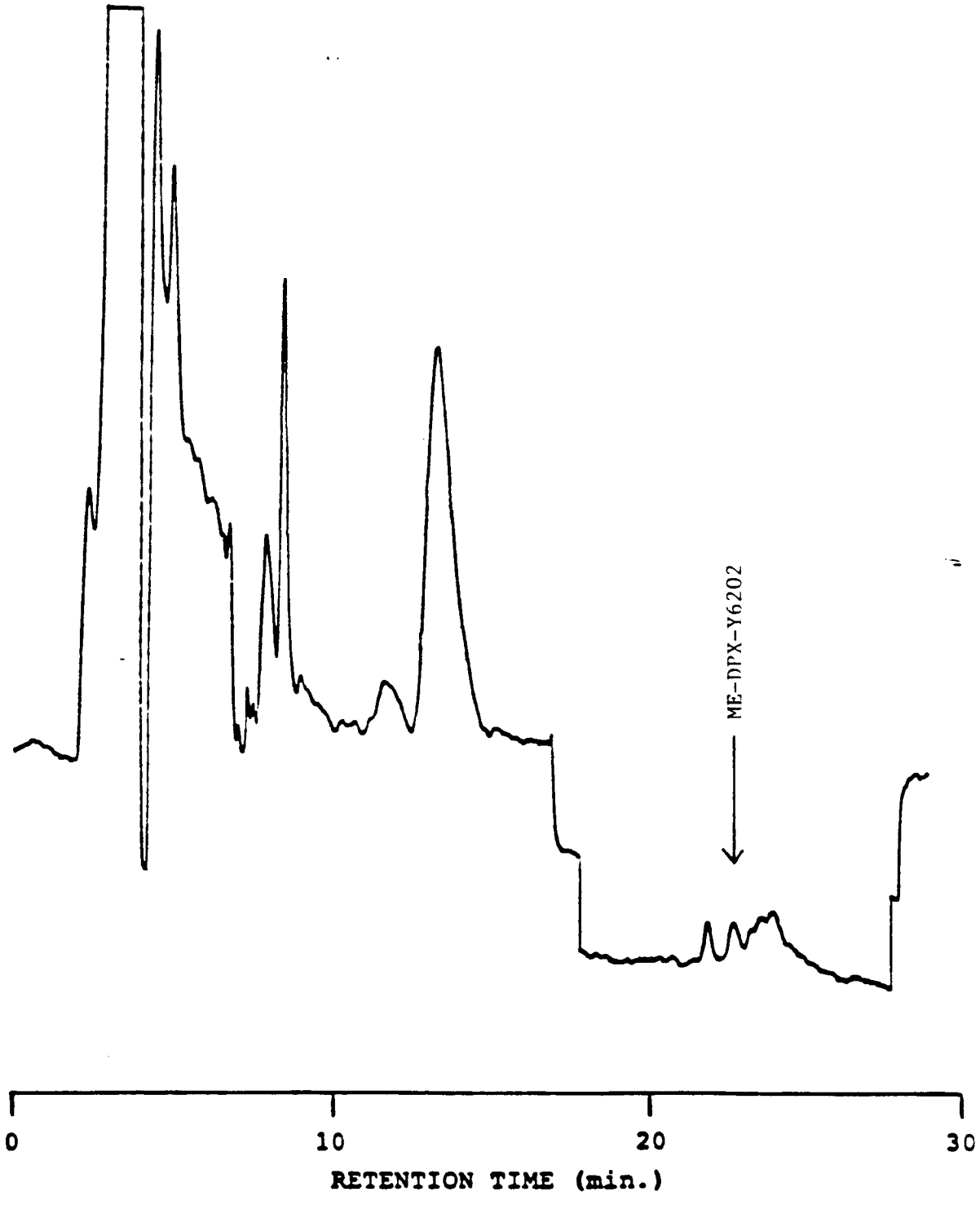


FIGURE 21: Chromatogram of fraction B from a soybean crude oil sample from a processing study where the soybeans were treated at 8 oz ai/A with DPX-Y6202 and sampled 72 days later (DPX-Y6202 Acid <0.05 ppm).

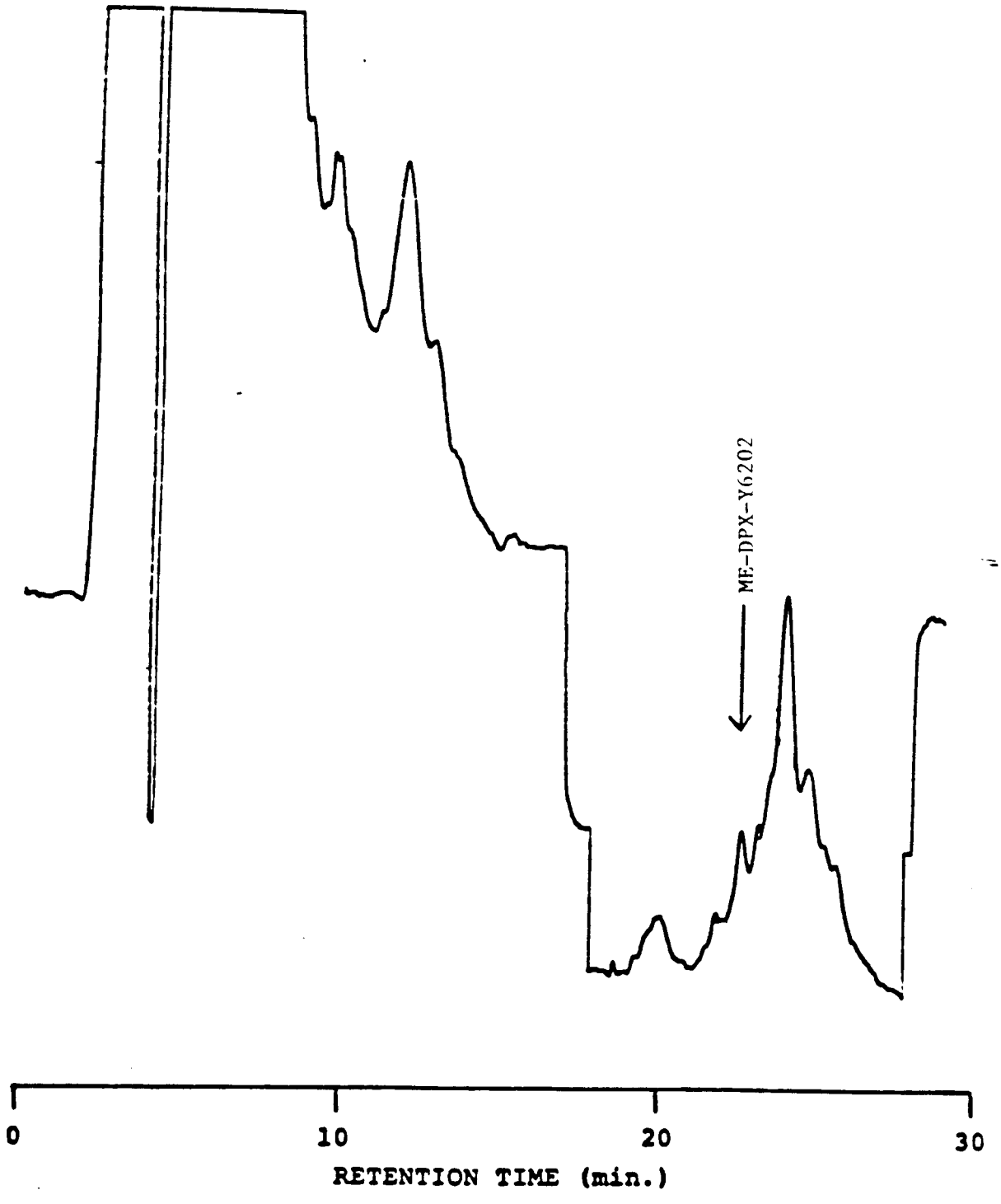


FIGURE 22. Chromatogram of fraction B from a control soybean soapstock sample from a processing study (DPX-Y6202 Acid <0.20 ppm). The final volume is 1.0 mL for the 4.0 gram sample.

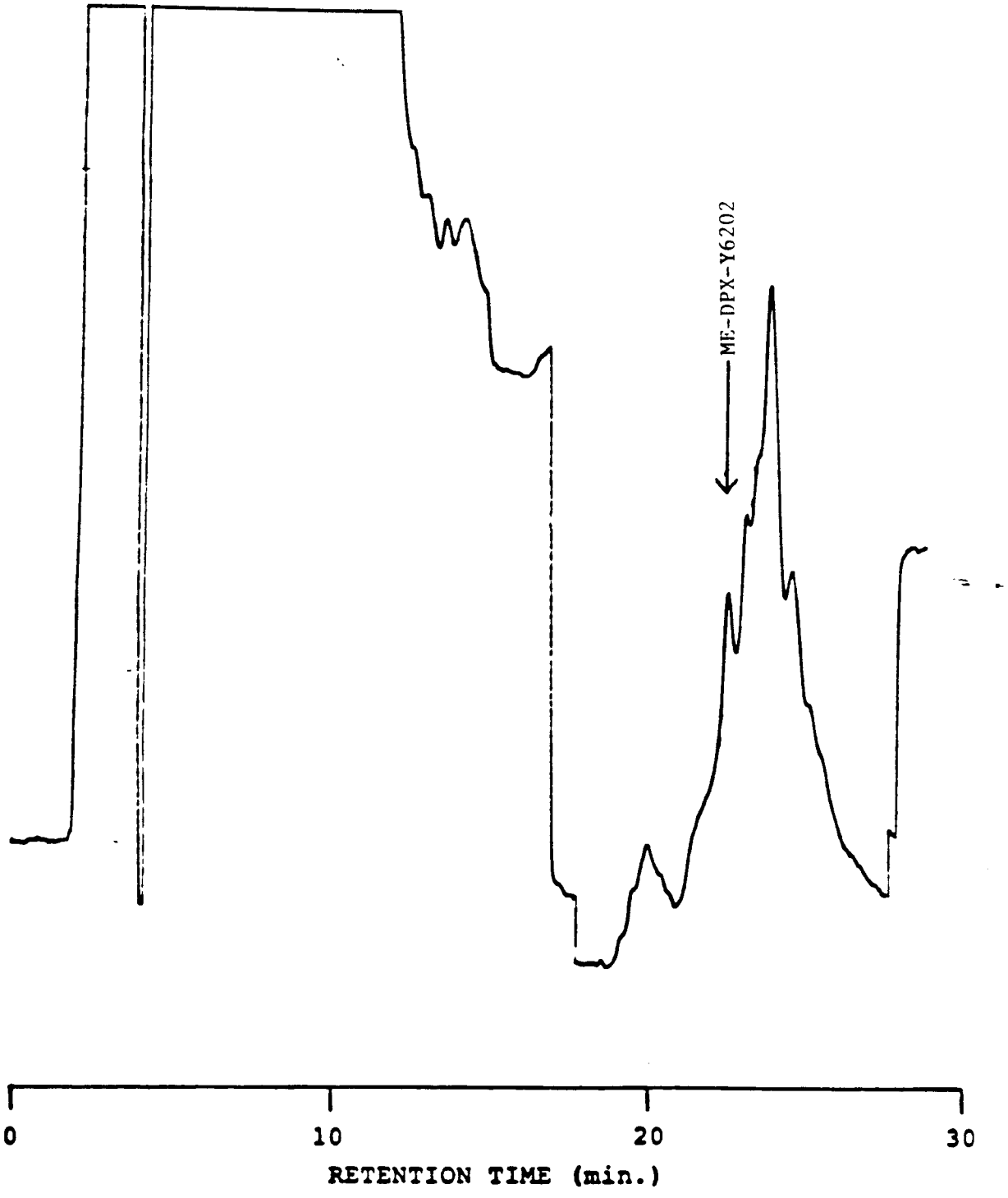


FIGURE 23. Chromatogram of fraction B from the same control soybean soapstock sample as in Figure 22 fortified with 0.05 ppm DPX-Y6202 and 0.05 ppm DPX-Y6202 Acid (0.096 ppm equivalent DPX-Y6202 Acid) (Recovery = 103% after subtraction of the background in the control). The final volume is 1.0 mL for the 4.0 gram sample.

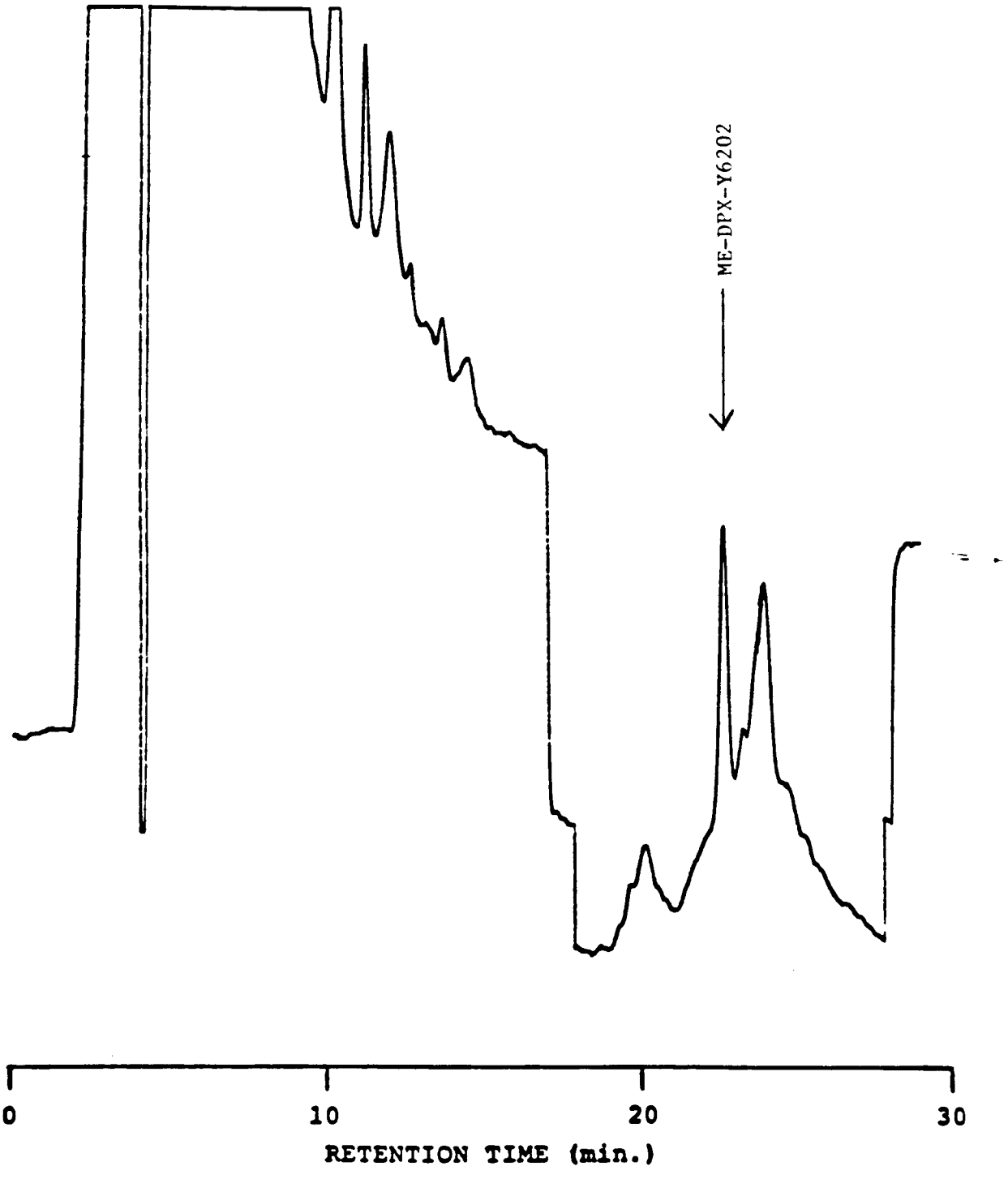


FIGURE 24. Chromatogram of fraction B from a soybean soapstock sample from a procesing study where the soybeans were treated at 8 oz ai/A with DPX-Y6202 and sampled 72 days later (DPX-Y6202 Acid = 0.25 ppm). The final volume is 1.0 mL for the 4.0 gram sample.

APPENDIX I

DESCRIPTION OF MPLC

A schematic of the MPLC is shown in Figure 2. It is composed of a Rainin model 653 intermediate pressure pump, a Lobar® silica column, a Valco 16 loop electronically activated valve, a Valco injection valve, a Foxy® fraction collector, and a Foxy® accessory controller. When samples are being processed the mobile phase is pumped at 5.0 mL/min to the injection valve, to the 16 loop valve, back to the injection valve and then to the column. The effluent from the column then goes to the fraction collector where it can be directed either to waste or else to 50 mL centrifuge tubes for collection of sample fractions.

The collection of sample fractions containing DPX-Y6202 and DPX-Y6202 Acid is controlled as a function of time by the fraction collector. After a sample has been processed, the fraction collector, through the accessory controller, recycles the time program to the beginning and steps the 16 loop valve to the next loop for injection of the next sample.

For each sample injected, the fraction collector is set to dump the column effluent to waste until DPX-Y6202 starts to elute from the column. When it starts to elute, the fraction collector moves to the first 50 mL centrifuge tube and collects

the effluent there. After the DPX-Y6202 elutes, the fraction collector moves back to the dump position until the DPX-Y6202 Acid starts to elute. At this time it moves to the second 50 mL centrifuge tube and collects the effluent there. After the DPX-Y6202 Acid elutes, the fraction collector moves back to the dump position until the rest of the sample has been eluted at which time the next injection is made.

APPENDIX II

ANALYSIS PROCEDURE FOR SOYBEANS

A manually operated medium-pressure LC could also be used tc clean-up samples rather than the automated system described in the procedure. Each sample would be loaded, injected, and the fractions containing DPX-Y6202 and DPX-Y6202 collected before the next sample would be processed.

Equipment

The manual system would be constructed from a medium or high-pressure LC pump capable of delivering 5.0 mL/min. of solvent and an HPLC injection valve fitted with a 2.0 mL sample loop. The column used is the same one described in the main procedure. Some type of stopwatch or timer would also be needed to time collection of the two peaks.

Operation

Calibration. To calibrate the system use 4 mL of a standard containing 1.0 µg/mL each of DPX-Y6202 and DPX-Y6202 Acid in solution E to load the sample loop. Then inject the standard and discard the column effluent for the first ten minutes. Then collect thirty 1.0 min fractions of the column effluent in 13 mL glass-stoppered centrifuge tubes and evaporate the fractions to dryness with nitrogen. Redissolve each fraction in 1.0 mL of solution B and analyze by HPLC for both DPX-Y6202 and DPX-Y6202 Acid

at the same time by using both columns in series and solution B as the mobile phase. Calculate the time needed to collect the DPX-Y6202 and DPX-Y6202 Acid peaks and use these times to collect the fractions during analyses of samples.

Sample Analysis. To analyze samples, load a sample in the injection loop and inject the sample. Then collect the DPX-Y6202 and DPX-Y6202 Acid fractions based on a recent calibration of the column in separate 50 mL glass-stoppered centrifuge tubes. Label the DPX-Y6202 fraction as fraction A and the DPX-Y6202 Acid fraction as fraction B. Twenty minutes after the DPX-Y6202 Acid fraction has been collected, load the next sample, inject it, and collect the two fractions. Continue until all samples have been processed.

Then evaporate the sample fractions to dryness with nitrogen and store them in a refrigerator at <4°C until they can be analyzed by HPLC.

APPENDIX III

MPLC SAMPLE LOADING PROCEDURE

1) Move the sample injection valve to the load position and step the 16-loop valve to the #2 position (See Figure 2).

2) Flush the #2 loop with 5 to 10 mL of solution E. Then load filtered sample #1 using about 1 mL of air first to purge the solution E from the loop.

3) Step the 16-loop valve to loop #3 before removing the syringe. Then rinse loop #3 with 5 to 10 mL of solution E and load sample #2 as described in step #2.

4) Continue loading the rest of the samples (up to a maximum of 15) as described in step #3.

5) After the last sample, step to the next loop and load a standard mixture of DPX-Y6202 and DPX-Y6202 Acid in solution E to check that the calibration of the column is correct.

6) After the standard has been loaded, step to the next loop, remove the syringe, and rinse the loop with 10 mL of solution E.

7) Before removing the syringe step the 16-loop valve to the #1 position and also fill that loop with solution E. Switch the injection valve to the run position and remove the syringe used to rinse with solution E.

8) Push the run button on the fraction collector to switch the valve to the #2 loop and inject the first sample.

APPENDIX IV

CALIBRATION OF MPLC

1) Use 4 mL of a standard containing 1.0 ug/mL each of DPX-Y6202 and DPX-Y6202 Acid in solution E to load loop #1 of the MPLC as described in Appendix B.

2) Load loop #2 with solution E, then step the valve to loop #3 and fill with solution E. Step the valve to loop #1, fill with solution E, and switch the injection valve to the run position as described in Appendix B.

3) Program the fraction collector to discard 10 minutes of effluent to waste and then collect 30 1.0 minute fractions in either 13 mL or 50 mL glass-stoppered centrifuge tubes.

4) Evaporate the samples to dryness, redissolve in solution B, and analyze by HPLC for both DPX-Y6202 and DPX-Y6202 Acid. Both compounds can be analyzed at the same time by using both columns in series and solution B as mobile phase.

5) Calculate the times needed to collect the DPX-Y6202 and DPX-Y6202 Acid and program these times into the fraction collector. A typical timing program is given in ~~Table 1.~~ Table 2

STORAGE LOCATION OF RAW DATA, REPORTS

E. I. du Pont de Nemours and Company, Inc.
Agricultural Products Department
Experimental Station
Residue Studies Groups' Archives
Wilmington, Delaware 19898