B-88-11

403224-03

Study Title

DETERMINATION OF DPX-Y6202, DPX-Y6202 ACID, AND ME-DPX-Y6202 RESIDUES IN TISSUES

Data Requirement

U.S. EPA Pesticide Assessment Guidelines
Subdivision 0, 171-4

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Study Completed On

May 5, 1987

Performing Laboratory

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Laboratory Project ID

AMR-627-86

72A

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 August 14, 1987

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.

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ABSTRACT

A procedure has been developed for the analysis of DPX-Y6202, DPX-Y6202 Acid, and ME-DPX-Y6202 in cow and chicken tissues. Tissue samples were extracted with methanol which was then evaporated. The oily residue was then hydrolyzed with a mixture of lipase and esterase enzymes to disassociate the fat and also convert DPX-Y6202 and ME-DPX-Y6202 to DPX-Y6202 Acid. The DPX-Y6202 Acid was then extracted from the aqueous enzyme solution with chloroform, and cleaned up on a silica cartridge. The level of DPX-Y6202 Acid was determined by multi-dimensional HPLC with spectrophotometric detection at 335 nm. Recoveries averaged 80% for DPX-Y6202 and 85% for DPX-Y6202 Acid with detection limits of 0.02 ppm for muscle and 0.05 ppm for kidney and liver for both compounds.

INTRODUCTION

Ethyl-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy] propanoate, (DPX-Y6202), is the active ingredient in Assure® Herbicide. DPX-Y6202, with the common name, quizalofop ethyl, is effective in controlling grasses in broadleaf crops such as soybeans. Because soybeans may be used in cattle and poultry feed, we have developed a procedure to determine residues of DPX-Y6202 and its metabolites in cattle and chicken tissues. DPX-Y6202 has been shown by Hunt (1) and Hundley (2) to be metabolized by chickens and goats to DPX-Y6202 Acid and to the methyl ester of DPX-Y6202 (ME-DPX-Y6202). The

structures of DPX-Y6202 (ethyl-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy] propanoate), DPX-Y6202 Acid ([4-(6-chloro-quinoxalin-2-yloxy)phenoxy] propanoic acid), and ME-DPX-Y6202 (methyl-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy] propanoate) are given in Figure 1.

We have developed a procedure to determine these three compounds in tissues. Both DPX-Y6202 and ME-DPX-Y6202 are converted to DPX-Y6202 Acid during the procedure and the DPX-Y6202 Acid measured by multi-dimensional HPLC.

PROCEDURE

Equipment and Reagents

A Du Pont Model 8800 HPLC (E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware) consisting of microprocessor controller, Model 870 pump, a column oven, and a data system was used. The column oven was fitted with 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). Samples were injected with a Hitachi Model 655A-40 autosampler (EM Science, Gibbstown, New Jersey). The detector was a Waters Model 481 (Waters Associates, Milford, Massachusetts) spectrometer set at 335 nm. The HPLC columns were a Sepralyte® C2, 4.6 mm x 15 cm, column (Analytichem International, Harbor City, California) and a Zorbax® Phenyl, 4.6 mm x 15 cm, column (E. I. du Pont de Nemours and Company).

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 (Fisher Scientific Company, Pittsburgh, Pennsylvania) was used for mixing of samples in centrifuge tubes. An International Equipment Company Model K centrifuge (Fisher Scientific), fitted with a head to hold six 250 mL centrifuge jars, and an International Equipment Company Clinical Centrifuge, fitted with a head to hold four 13-mL centrifuge tubes were used to centrifuge samples. A Precision Model 50 shaking water bath (GCA Corporation, Precision Scientific Group, Chicago, Illinois) was used to shake the samples at 37°C for the enzyme hydrolysis step.

For concentration of samples, a vacuum rotary evaporator with a water bath set at 50°C was used. Either 250 mL glass-stoppered erlenmeyer flasks or pear-shaped flasks with 24/40 ground-glass joints (Kontes, Vineland, New Jersey), #K-608700, were used on the rotary 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 0.45 µm Durapore® filter, #HVLP 047 00, was used to filter the HPLC solvents (Millipore Corporation, Bedford, Massachusetts). Millipore Millex®-HV disposable Durapore® filters, #SJHV LO4 NS, were used to filter samples before they were injected on the HPLC.

Silica cartridges were used to clean up samples. Each cartridge was made from a Bond Elut® 75 mL reservoir fitted with two

20 µm pore size frits (Analytichem International). Each cartridge was packed with 10 grams of Adsorbosil®-LC silica, 200/425 mesh (Alltech Associates, Inc., Deerfield, Illinois).

The lipase enzyme from Rhizopus Arrhizus, #437706, was purchased from Behring Diagnostics, La Jolla, California in bottles containing 100,000 units. The esterase enzyme from porcine liver, #E3128, was purchased from Sigma Chemical Co., St. Louis, Missouri in bottles containing a 30 mg suspension in 3.2 M ammonium sulfate, pH 8. The activity of the enzyme was 260 units/mg and was dissolved in 2.8 mL of solution. Both enzymes were stored at 0 to 4°C until they were used.

The standards of DPX-Y6202 (purity = 99.7%), and DPX-Y6202 Acid (purity = 97.7%) were obtained from the Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware. All solvents were distilled-in-glass HPLC grade obtained from Fisher Scientific. The chloroform was stabilized with 0.75% ethanol. All other chemicals were A.C.S. reagent grade obtained from either Fisher Scientific or VWR Scientific, San Francisco, California.

Buffer Z was made by dissolving 12.6 grams of trizma hydrochloride (Sigma Chemical Co., St. Louis, Missouri), 0.4 grams of cholic acid (Sigma Chemical Co.), and 11.8 grams of calcium chloride dihydrate in 2.0 L distilled water. The pH was then adjusted, by monitoring with a pH meter, to 7.8 with 1 N sodium hydroxide. Buffer Y was made by dissolving 105.7 grams of ammonium

sulfate and 0.44 gram of dibasic potassium phosphate in 250 mL distilled water. The pH of this buffer was adjusted by monitoring with a pH meter to 6.0 with concentrated phosphoric acid.

A lipase enzyme solution was then made by adding 2.7 mL of buffer Y to a 100,000 unit bottle of lipase. The bottle was rolled gently to suspend the enzyme and was stable for two weeks after it was suspended in the buffer as long as it was stored at 0 to 4°C.

The two HPLC mobile phases were made by mixing together the proportions in Table 1 of acetonitrile and pH 2.2 phosphoric acid solution. The pH 2.2 phosphoric acid solution was made by adjusting the pH of distilled water to 2.2 with concentrated phosphoric acid. The pH was monitored with a pH meter. The prepared mobile phases were then filtered with a 0.45 µm Durapore® filter before using. A pH 8.5 solution was made by adjusting the pH of 500 mL distilled water to 8.5 ± 0.5 using 1 N NaOH. Other solutions were made in the proportions given in Table 3.

Isolation and Clean Up

Each tissue sample was chopped in a Quizinart food processor while partly frozen, and then return to the freezer. A 10 gram aliquot of each sample was cut from the frozen chopped sample and weighed into a 250 mL glass centrifuge bottle and the rest of the sample returned to the freezer. 150 mL methanol was added, the sample homogenized for 1 min. with the Tissumizer® homogenizer, and the shaft and generator rinsed well with methanol which was collected in the centrifuge bottle. After centrifuging

for 10 min., the liquid was decanted into a 250 mL glass-stoppered erlenmeyer flask, and the volume reduced to about 30 mL on the rotary evaporator.

The tissue solids in the centrifuge bottle were re-extracted with 120 mL of methanol as described above. The extract was added to the erlenmeyer flask with the remaining solution from the first extraction and the sample then concentrated to dryness on the rotary evaporator. Since tissue samples tend to foam near dryness, they were removed when foaming started and evaporated to dryness with a stream of nitrogen. 100 mL of buffer Z was added to each sample in the erlenmeyer flask and it was shaken and ultrasonically mixed to emulsify the fat. After the fat was emulsified, the pH of each sample was checked with a pH meter and adjusted to 7.8 ± 0.1 with 1 N NaOH. After the pH was adjusted, 7500 units of the lipase enzyme solution (200 µL) and 300 units of the esterase solution 115 µL) was added. The samples were then incubated on the shaker bath for at least 12 hours at 37°C. The shaking action was adjusted to 100-120 shakes/minute.

After the samples had incubated for at least 12 hours, the contents of the erlenmeyer flask was transferred to a 500 mL separatory funnel and 5 mL of 10% hydrochloric acid added. Each erlenmeyer flask was then rinsed twice with 25 mL of acetonitrile each time. Each rinse was ultrasonically mixed and shaken in the flask and then also transferred to the separatory funnel. An additional 150 mL rinse of the erlenmeyer flask with chloroform was made and this also transferred to the separatory funnel. After the

contents were shaken vigorously for 1 minute and the two layers had separated, the bottom layer was drained into a 500 mL pear-shaped flask and then concentrated to dryness on a rotary evaporator at 50°C. The top layer in the separatory funnel was discarded.

Each sample was then transferred from the pear-shaped flask to a 13 mL centrifuge tube with 3 x 3 mL rinses of solution A (see Table 3). The rinses were ultrasonically mixed and shaken as needed to remove the residue off the walls of the pear-shaped flask. The solvent was then evaporated from each sample in the centrifuge tube using the N-EVAP® nitrogen evaporator with the water bath at room temperature.

For each sample, a 20 µm pore size frit was placed in the bottom of a 75 mL reservoir. Ten grams of Adsorbosil® silica was slurried with 50 mL of 2-propanol in a 150 mL beaker and then added to the reservoir. The beaker was rinsed with enough 2-propanol to completely transfer the silica to the reservoir. After the silica had settled, another frit was placed snuggly on top of the silica. Care was taken to avoid trapping air under the frit. After the 2-propanol had drained to the frit, each cartridge was rinsed successively with 50 mL of solution A and 100 mL of solution F (see Table 3). The cartridge did not run dry since the flow stopped whenever the solvent drained to the top frit.

Each sample was transferred from the centrifuge tube to the cartridge using 3 x 3 mL rinses with solution F. The cartridge was then rinsed with 25 mL of solution F which was discarded. DPX-Y6202

Acid was eluted with 65 mL of solution E which was collected in a 100 mL pear-shaped flask.

Each sample was then concentrated to dryness on a rotary evaporator at 50°C. The residue for each sample was then transferred to a 13 mL glass-stoppered centrifuge tube with 3 x 3 mL rinses of the pear-shaped flask with solution A. Again, the rinses were ultrasonically mixed and shaken as needed to remove the residue off the walls of the pear-shaped flask. The solvent was then evaporated from each sample in the centrifuge tube using the N-EVAP® nitrogen evaporator with the water bath at room temperature. Each sample was then stored at 0 to 4°C until it was prepared for analysis on the HPLC.

Liquid Chromatography

Samples were quantitated by multi-dimensional HPLC using a combination of two columns. A diagram of the columns and switching valve arrangement is shown in Figure 2 where the first column (C_1) was the Sepralyte® C_2 column and the second column (C_2) was the Zorbax® Phenyl column. In valve position I, 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 the valve, to C_2, back to the valve, and then to the detector.

Table 2 gives a typical timing sequence for analysis of samples. At the time of injection, the valve was in position I and C_2 was bypassed. When DPX-Y6202 Acid started to elute from C_1, the valve was switched to position II to trap the peak on C_2. After the

peak was trapped, the valve was switched back to position I. The valve switching times were set at -0.20 and +0.05 min. around the retention time for DPX-Y6202 Acid on C_1 which was determined each morning.

After DPX-Y6202 Acid had been trapped on C_2, the mobile phase was changed from solution I to solution II and the flow rate increased from 1.0 mL/min. to 2.5 mL/min. to quickly equilibrate C_1 to the new mobile phase and to clean the rest of the sample off. After C_1 had equilibrated to the stronger mobile phase, the flow rate was decreased to 1.0 mL/min and the valve switched to position II to elute DPX-Y6202 Acid from C_2. After all peaks had eluted from C_2, the valve was switched to position I, the mobile phase changed to solution I, and the flow rate increased to 2.5 mL/min. to quickly equilibrate C_1 to solution I. After C_1 had equilibrated, the flow rate was decreased to 1.0 mL/min. and the next sample or standard injected.

The detector attenuation was set at 0.002 AUFS and the time constant at 5 seconds. The data system attenuation was set at 1, the oven temperature at 50°C, and the autosampler injection volume at 50 µL.

For HPLC analysis, each sample was redissolved in 5.0 mL of acetonitrile (2.0 mL for muscle), ultrasonically mixed, and vortex mixed. Then 5.0 mL of pH 8.5 solution (2.0 mL for muscle) was added and the sample vortex mixed for 30 seconds. After centrifuging for 5 minutes, 1.5 mL of the aqueous solution was then removed with a

pastuer pipette, transferred to a 2.0 mL disposable syringe, and filtered through the Millex® disposable filter into an autosampler vial. Samples were then injected on the HPLC intermixed with standards.

Standards

Stock standards. Stock standards for DPX-Y6202, and DPX-Y6202 Acid were each made at 100 µg/mL by dissolving 10 mg of each in 100 mL of solution H (see Table 3).

Fortifying standards. Intermediate stock standards of each were made at 10.0 µg/mL by diluting 5.0 mL of the stock standards to 50 mL with solution H. Fortifying standards at 1.0 µg/mL and 0.2 µg/mL were made by diluting 10.0 or 2.0 mL of the 10.0 µg/mL standards to 100 mL with solution H.

HPLC standards. A 10.0 µg/mL standard was made by placing 5.0 mL of the stock DPX-Y6202 Acid standard in a 50 mL volumetric flask, evaporating the solvent, and diluting to volume with solution II (see Table 1). Standards at 1.0, 0.5, 0.2, and 0.1 µg/mL were made by pipetting 10.0, 5.0, 2.0, and 1.0 mL of the 10 µg/mL standard into 100 mL volumetric flasks and making to volume with solution II. A 0.05 µg/mL standard was made by pipetting 5.0 mL of the 1.0 µg/mL standard into a 100 mL volumetric flask and making to volume with solution II. The 0.05, 0.1, and 0.2 µg/mL standards were then injected on the HPLC intermixed with the samples.

Calculations

The sensitivity for each standard injected on the HPLC, S, in (mm-mL)/µg units, was calculated by the equation;

$$S = \frac{P_S}{C_S} \qquad (1)$$

where, P_S was the peak height of the standard in millimeters and C_S was the concentration in µg/mL units. The average sensitivity for standards injected interspersed with samples, S_a, was calculated and used for calculation of sample concentrations.

The sample concentration, C, of DPX-Y6202 Acid, in µg/g units (ppm), was calculated using the equation;

$$C = \frac{(P)(V)}{(W)(S_a)} \qquad (2)$$

where, P was the sample peak height in millimeters, V was the final sample volume in mL, and W was the sample weight in grams. For samples fortified with DPX-Y6202, the concentration of DPX-Y6202 Acid was converted to DPX-Y6202 by multiplying by the molecular weight ratio of 1.08.

RESULTS AND DISCUSSION

Recovery data for tissue samples fortified with DPX-Y6202 and DPX-Y6202 Acid are given in Tables 4 and 5. Recoveries averaged 81% (s=10%) for cow tissue samples fortified with DPX-Y6202, 85% (s=9%) for cow tissue samples fortified with DPX-Y6202 Acid, 79% (s=7%) for chicken tissue samples fortified with DPX-Y6202, and 85% (s=4%) for chicken tissue samples fortified with DPX-Y6202 Acid. Samples were not fortified with ME-DPX-Y6202 since studies with milk (3) and eggs (4) had shown that recoveries for ME-DPX-Y6202 and DPX-Y6202 were not statistically different.

Chromatograms of control, fortified, and treated cow liver, kidney, and muscle samples are shown in Figures 3 to 11. Chromatograms of control, fortified, and treated chicken liver, kidney, and muscle samples are shown in Figures 12 to 20.

The extraction efficiency of methanol was validated by extracting two freeze-dried liver samples from a goat treated with [phenyl-^{14}C] DPX-Y6202 (P-label) and two freeze-dried liver samples from a goat treated with [quinoxaline-phenyl-^{14}C] DPX-Y6202 (QP-label) (2). Radioactivity in the methanol extract was determined by scintillation counting and in the remaining solids by combustion followed by scintillation counting. Methanol was able to extract 76% of the radioactivity from the liver of the P-label goat and 49% from the liver of the QP-label goat. These values compare to 79 to 85% for the P-label and 53 to 63% for the QP-label as reported by Hundley (2) for the same samples. He also showed that

acid, or base extraction and enzyme treatment did not release significantly more radioactivity from the goat liver.

The amount of cholic acid added to the enzyme hydrolysis step as an emulsifier is critical since too much inhibits the hydrolysis reaction. We have found that 0.01% as used in the milk procedure (3) and 0.02% as used in this and the egg procedure (4) both do not significantly inhibit the reaction but that 0.05% does. We ran the enzyme reaction overnight since that was convenient, but the reaction is probably completed in a shorter time.

Because of background interferences, a detection limit of 0.02 ppm is not practical for liver or kidney samples. Therefore, the detection limit for those matrices is 0.05 ppm. There are fewer interferences for muscle tissues so that a 0.02 ppm detection limit is practical. Because of fewer interferences for muscle samples, HPLC switching times of -0.35 and +0.10 minutes can be used as is used for the egg and milk procedures. This longer trapping time makes the response factor much less suspectible to minor retention time drifts during analysis of a series of samples.

REFERENCE

1) Hunt, O. R., "The Excretion and Metabolism of [Quinoxaline-phenyl $^{14}C(u)$] DPX-Y6202 in Laying Hens," E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, Agricultural Products Department, Document No. AMR-619-86.

2) Hundley, S. G., "Metabolism of ^{14}C-Labeled DPX-Y6202 by Lactating Goats," E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, Agricultural Products Department, Document No. AMR-618-86.

3) Hershberger, L. W., "Determination of DPX-Y6202, DPX-Y6202 Acid, and ME-DPX-Y6202 Residues in Bovine Milk," E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, Agricultural Products Department, Document No. AMR-515-86, Revision A.

4) Hershberger, L. W., "Determination of DPX-Y6202, DPX-Y6202 Acid, and ME-DPX-Y6202 Residues in Eggs," E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, Agricultural Products Department, Document No. AMR-623-86.

TABLE 1

AQUEOUS SOLUTION COMPOSITIONS

Solution	Percent Acetonitrile	Percent pH 2.2 Phosphoric Acid
I	40	60
II	50	50

TABLE 2

TYPICAL HPLC TIMING SEQUENCE

Time Range (min)	Mobile Phase	Flow Rate (mL/min)	Valve Position	Columns Used
0.00 to 6.80	Solution I	1.0	I	C_1
6.80 to 7.05	Solution I	1.0	II	$C_1 + C_2$
7.05 to 8.00	Solution I	1.0	I	C_1
8.00 to 18.00	Solution II	2.5	I	C_1
18.00 to 19.50	Solution II	1.0	I	C_1
19.50 to 30.00	Solution II	1.0	II	$C_1 + C_2$
30.00 to 43.00	Solution I	2.5	I	C_1

TABLE 3

ORGANIC SOLUTION COMPOSITIONS

Solution	Hexane	Acetone	Glacial Acetic Acid
A	---	980	20
E	580	400	20
F	780	200	20
H	---	999	1

TABLE 4

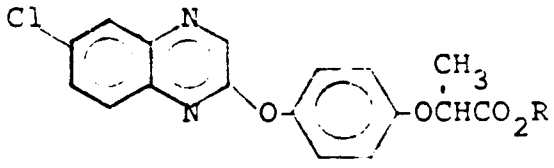
DPX-Y6202 RECOVERIES

Matrix Compound	Number of Samples	Spike Range (ppm)	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)
Chicken Kidney	2	0.05 - 0.10	76 - 86	81	---
Chicken Liver	3	0.05 - 0.10	74 - 80	78	3
Chicken Muscle	2	0.02 - 0.05	70 - 90	80	---
Cow Kidney	3	0.05 - 0.10	58 - 83	71	13
Cow Liver	4	0.05 - 0.10	74 - 86	81	5
Cow Muscle	5	0.02 - 0.10	79 - 97	86	7
Total	19	0.02 - 0.10	58 - 97	80	9

TABLE 5

DPX-Y6202 ACID RECOVERIES

Matrix Compound	Number of Samples	Spike Range (ppm)	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)
Chicken Kidney	3	0.05 - 0.10	80 - 88	85	5
Chicken Liver	2	0.05 - 0.10	78 - 86	82	---
Chicken Muscle	2	0.05 - 0.10	85 - 88	86	---
Cow Kidney	3	0.05 - 0.10	81 - 86	83	3
Cow Liver	4	0.05 - 0.10	64 - 96	81	13
Cow Muscle	4	0.02 - 0.10	84 - 100	90	7
Total	18	0.02 - 0.10	64 - 100	85	8



R = C_2H_5 for DPX-Y6202
R = H for DPX-Y6202 Acid
R = CH_3 for ME-DPX-Y6202

FIGURE 1: Structures of DPX-Y6202, DPX-Y6202 Acid, and ME-DPX-Y6202.

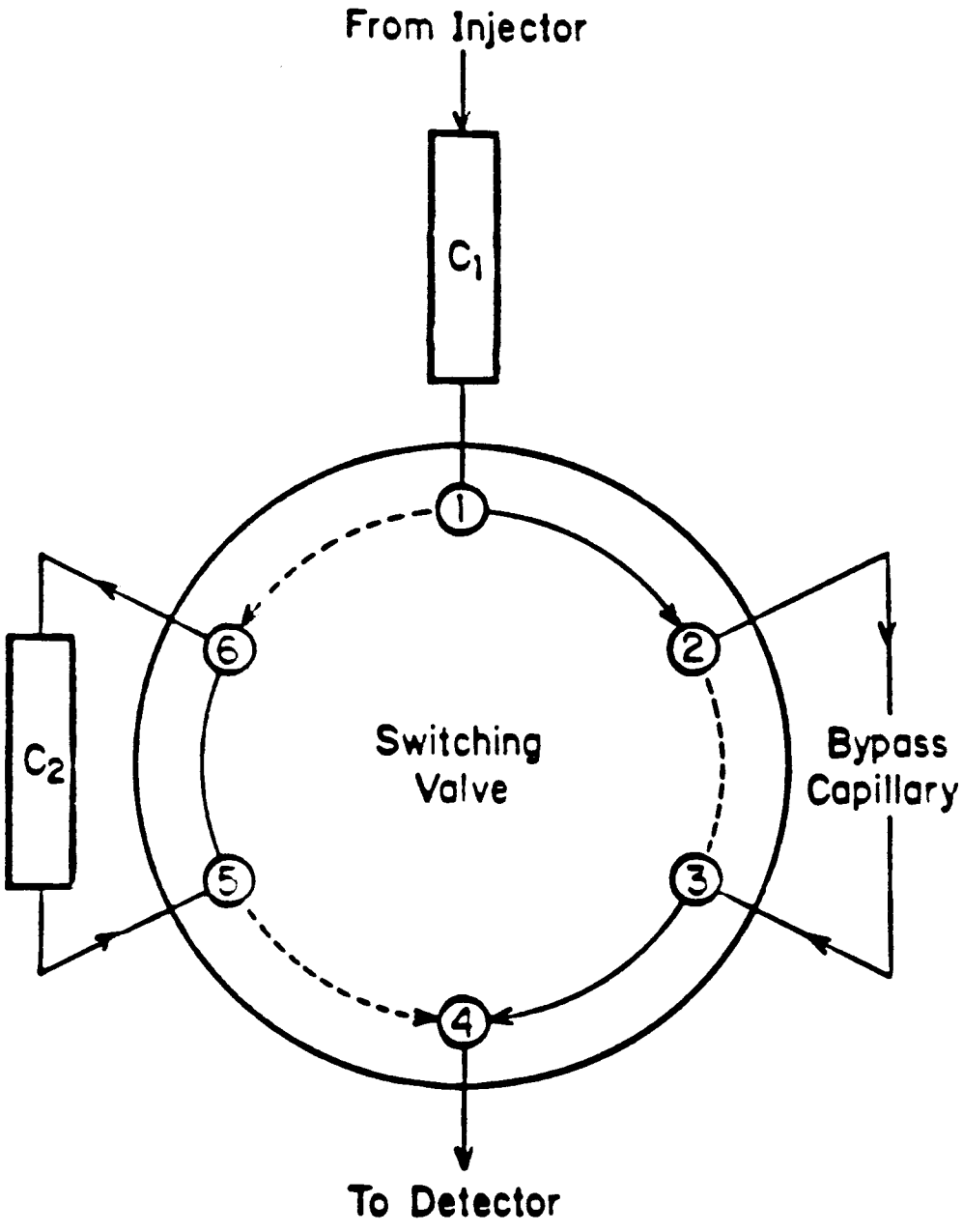


FIGURE 2: Chromatographic column and switching valve arrangement. Position I internal valve connections are designated by ___ and Position II by ----.

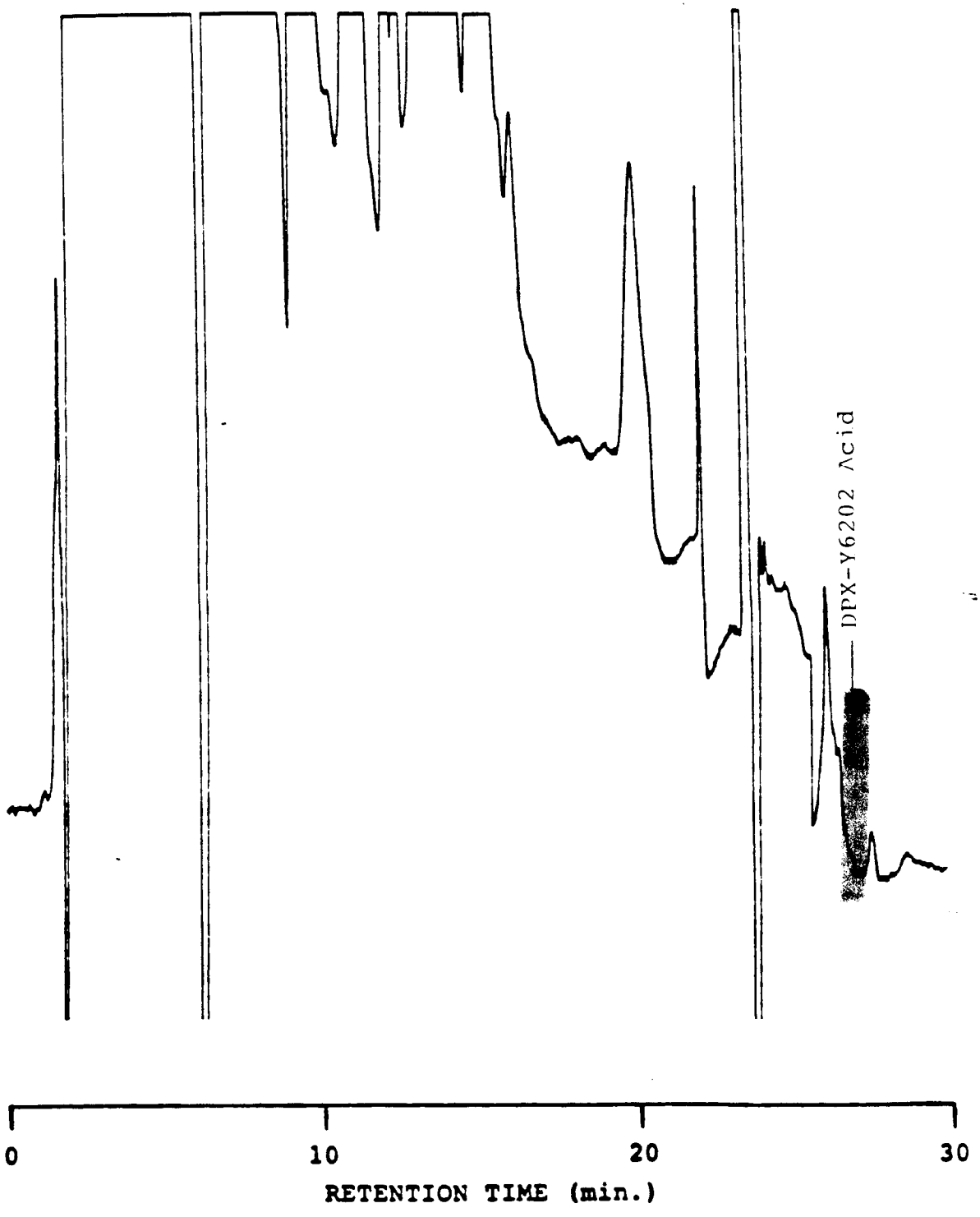


FIGURE 3: Chromatograms of a [redacted] sample.

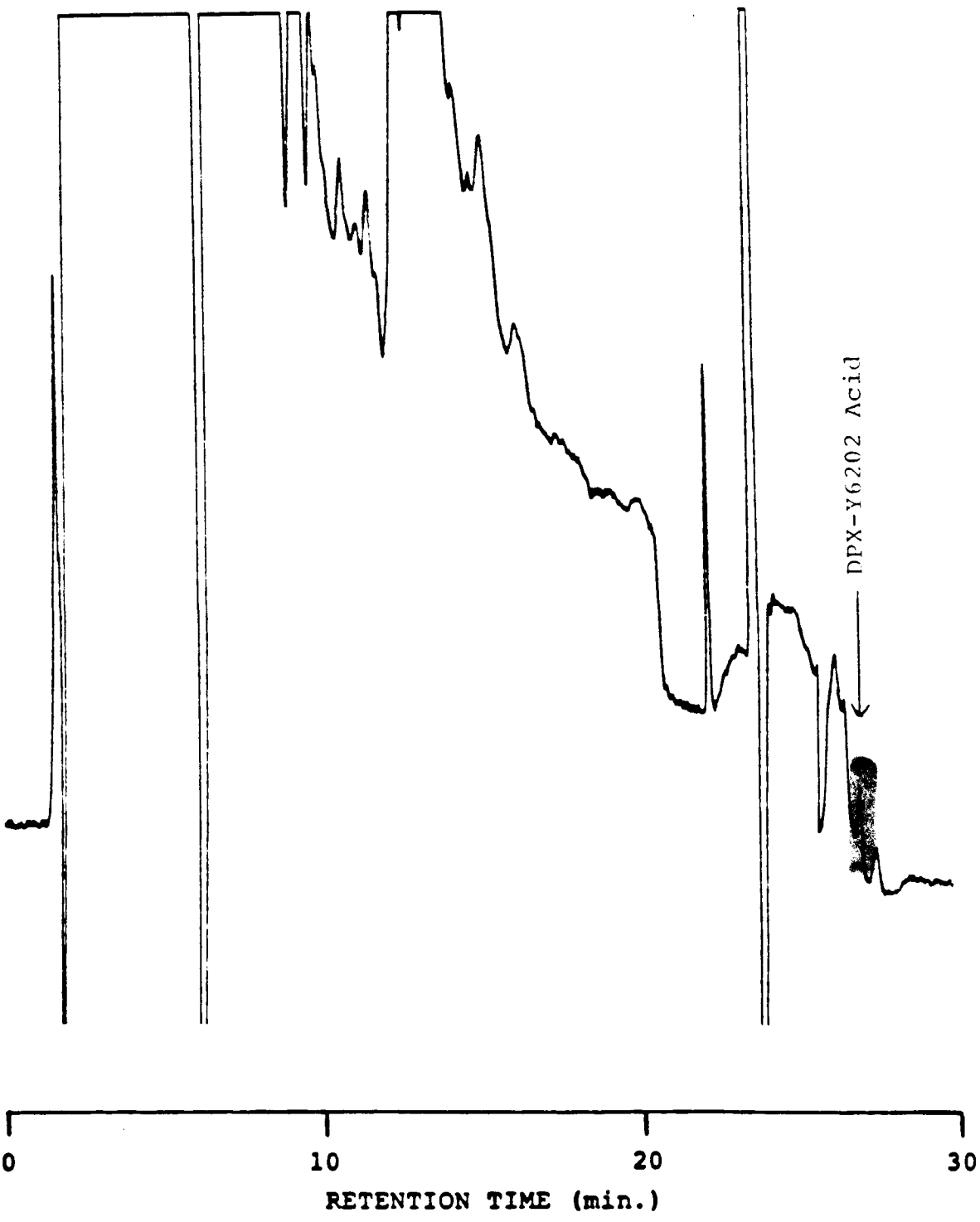


FIGURE 4: Chromatogram of the same control cow liver sample as in Figure 3 fortified wit[redacted]ppm DPX-Y6202 Acid (Recovery = 84%)

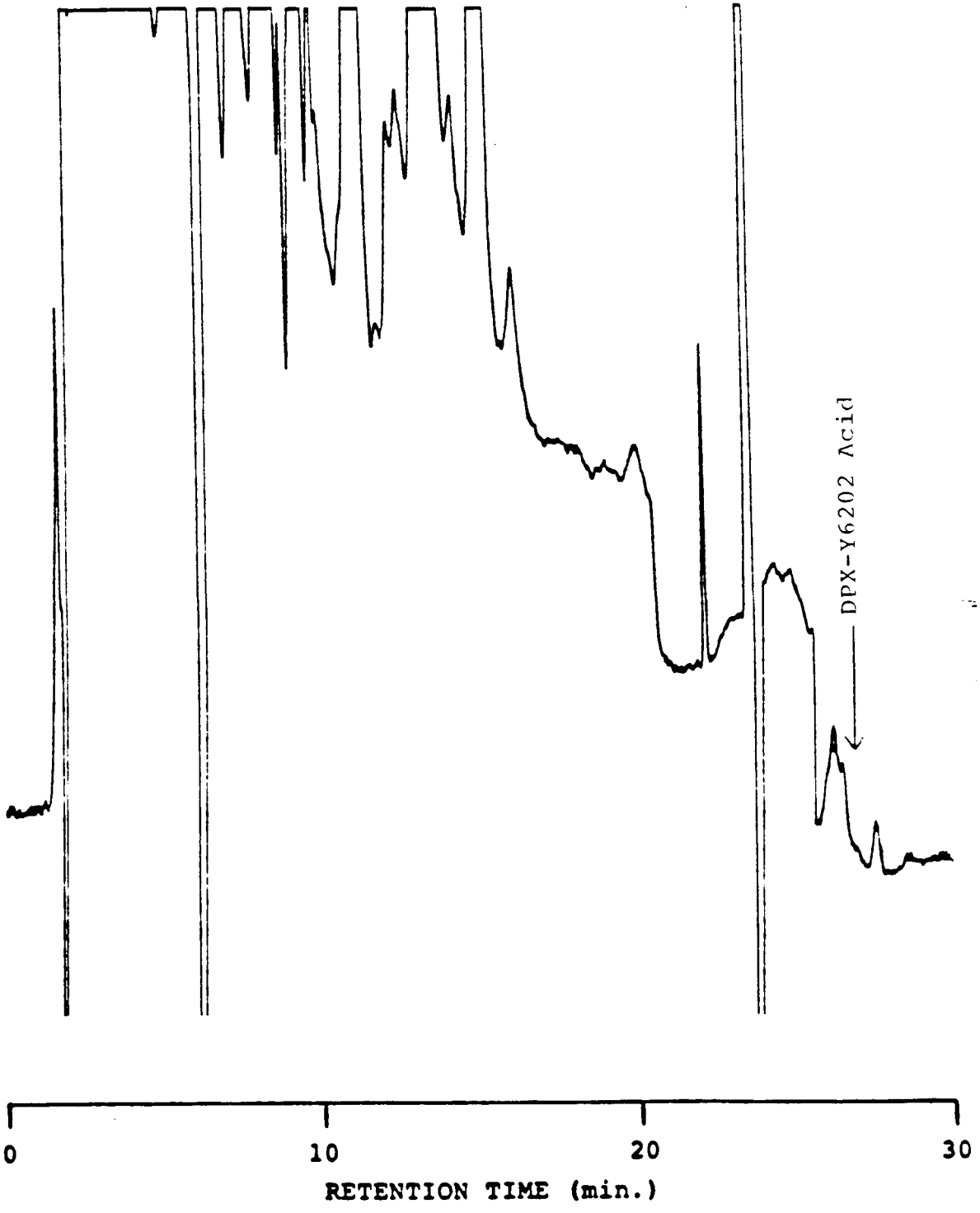


FIGURE 5: Chromatogram of a liver sample from cow #11 fed 5.0 ppm DPX-Y6202

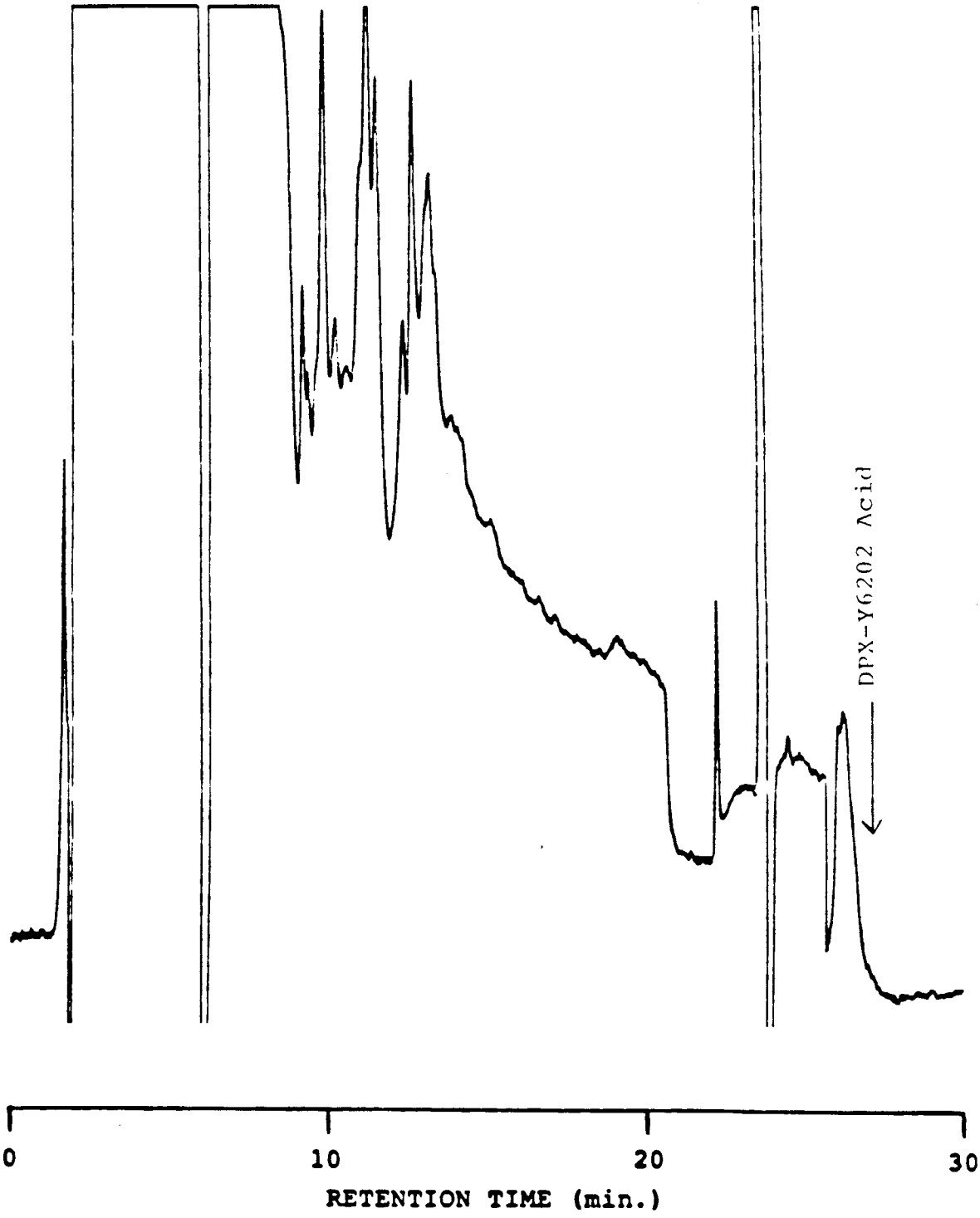


FIGURE 6: Chromatogram of a control cow kidney sample.

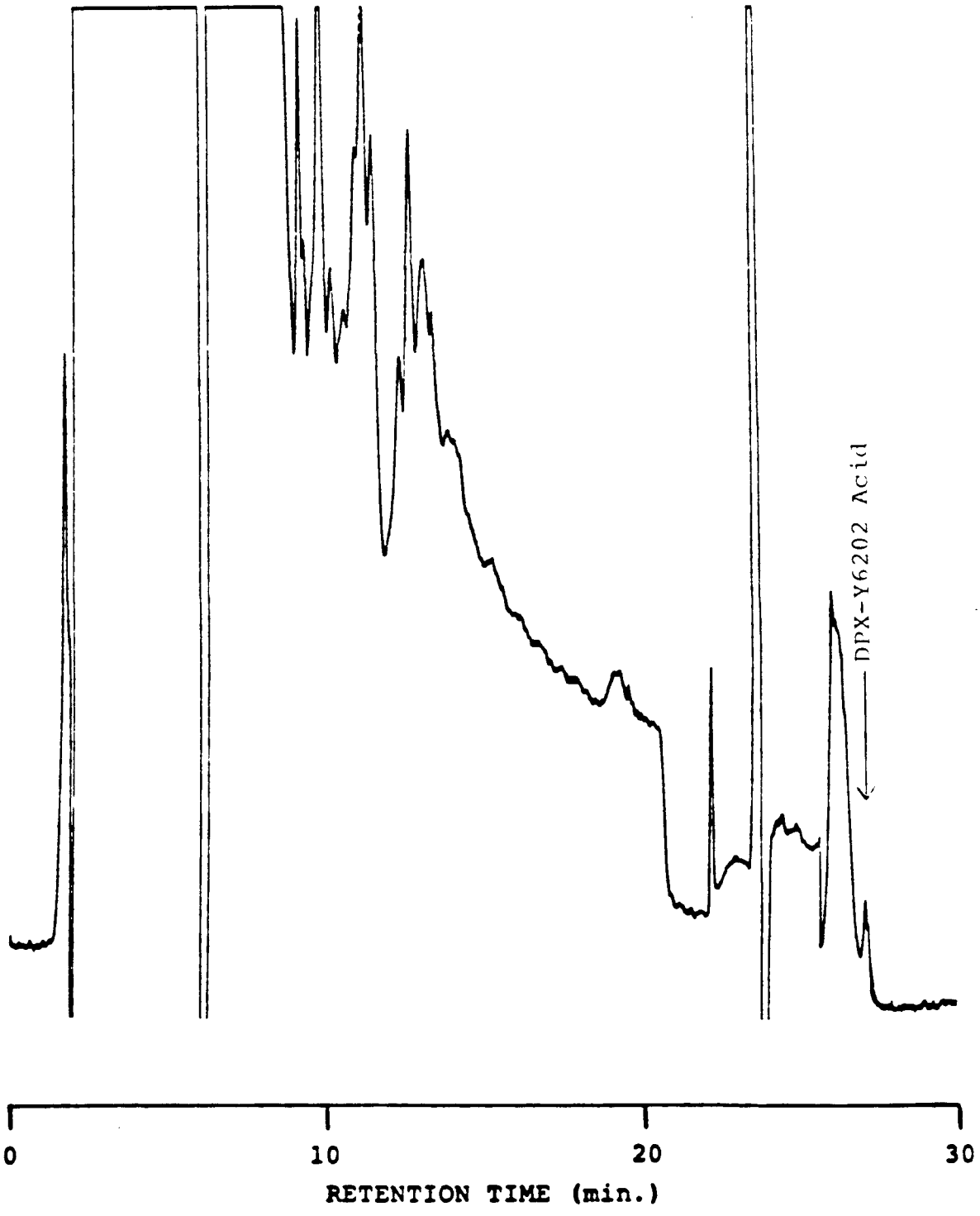


FIGURE 7: Chromatogram of the same control cow kidney sample as in Figure 6 fortified with 0.05 ppm DPX-Y6202 Acid (Recovery = 82%).

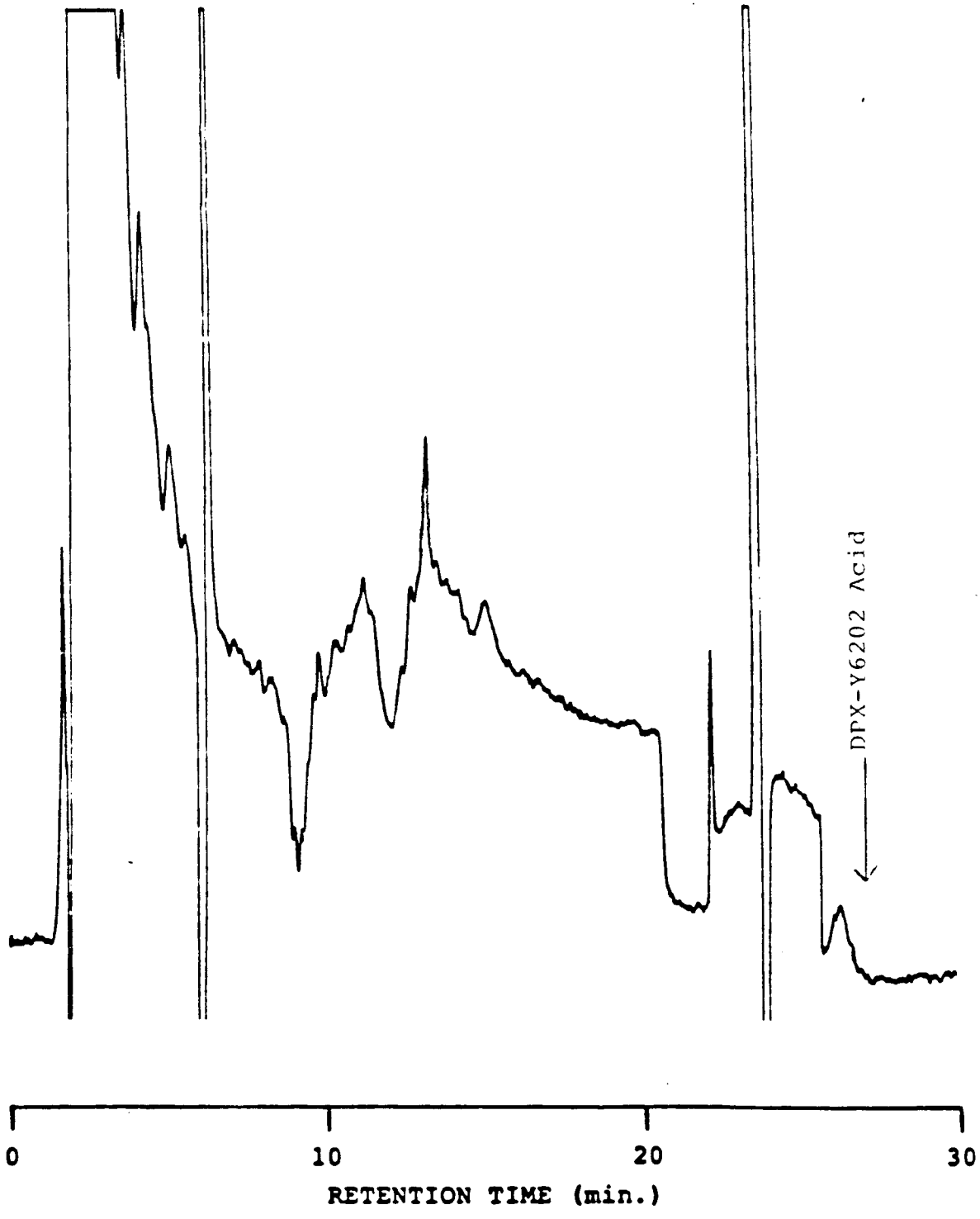


FIGURE 8: Chromatogram of a kidney sample from cow #12 fed 5.0 ppm DPX-Y6202.

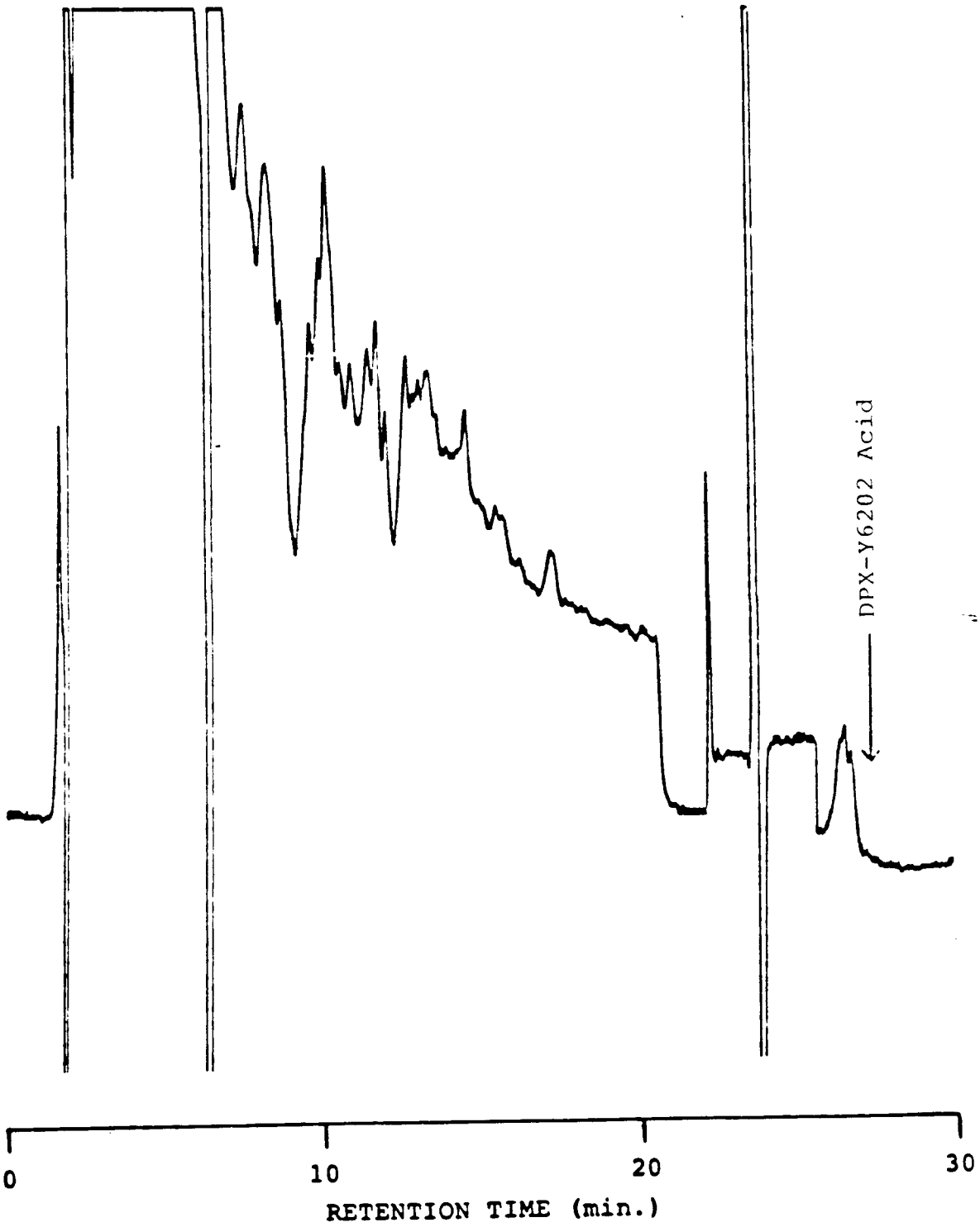


Figure 9: Chromatogram of a control cow skeletal muscle sample.

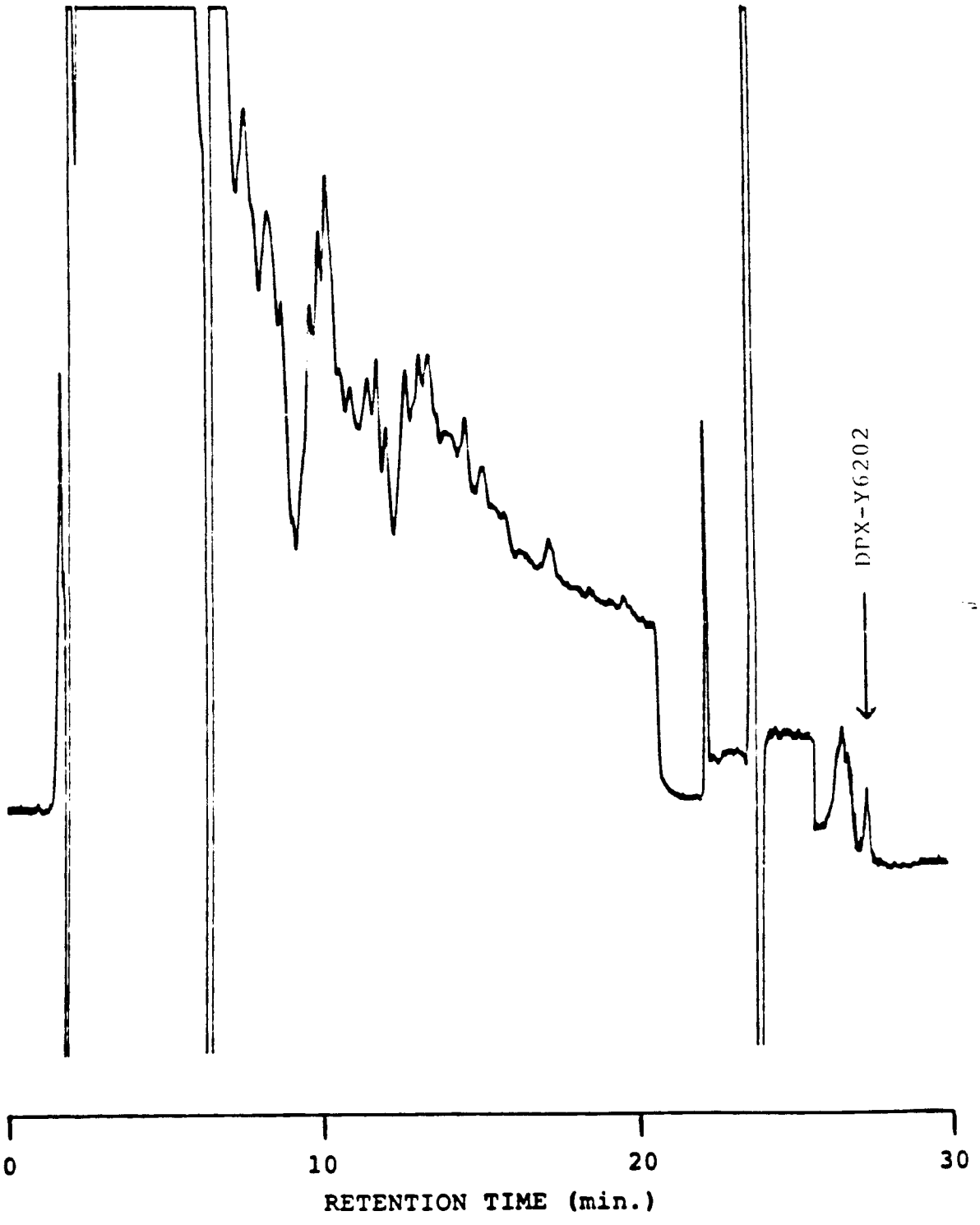


Figure 10: Chromatogram of the same control skeletal muscle sample as in Figure 9 fortified with 0.02 ppm DPX-Y6202 Acid (Recovery = 100%).

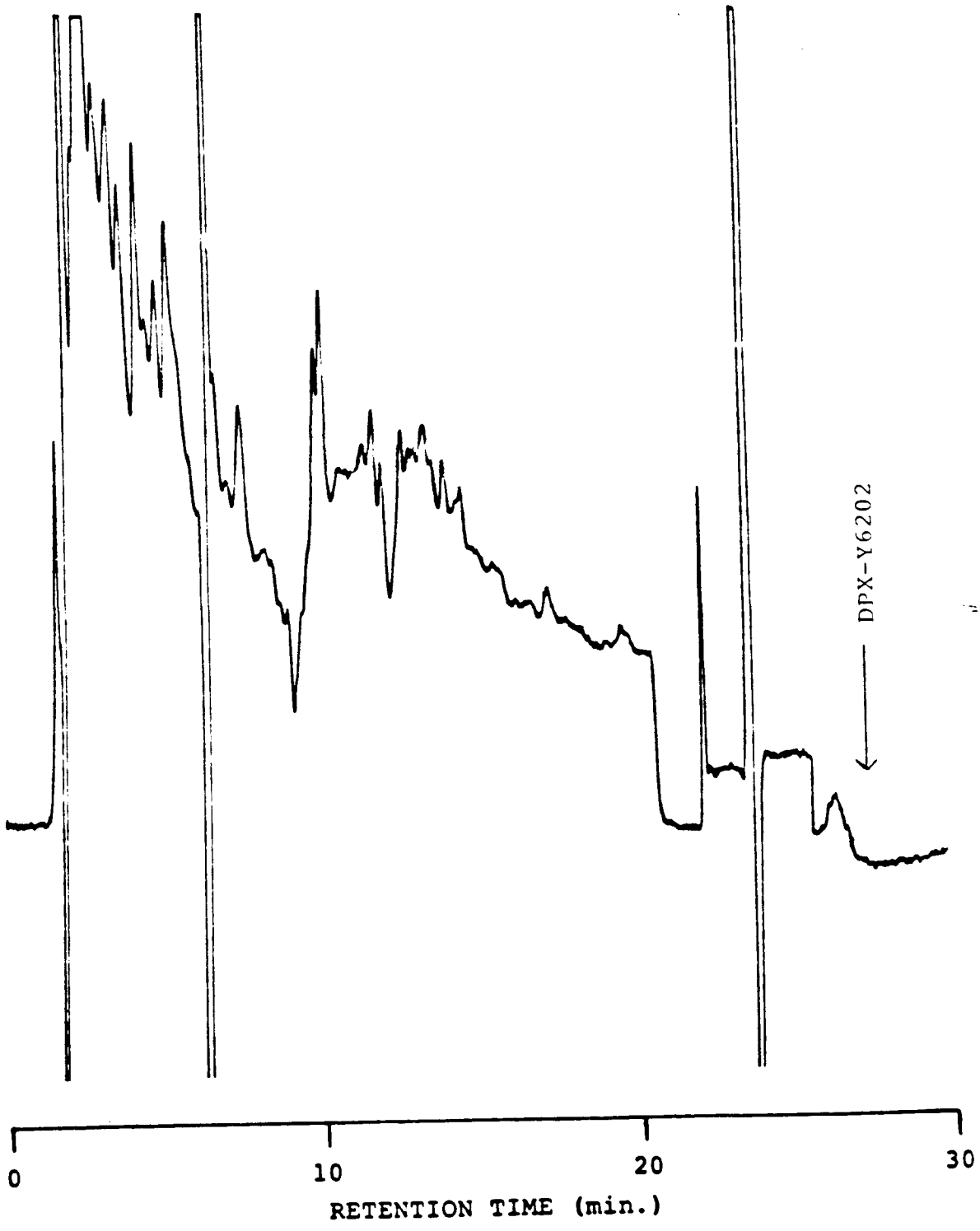


Figure 11: Chromatogram of a skeletal muscle sample from cow #11 fed 5.0 ppm DPX-Y6202.

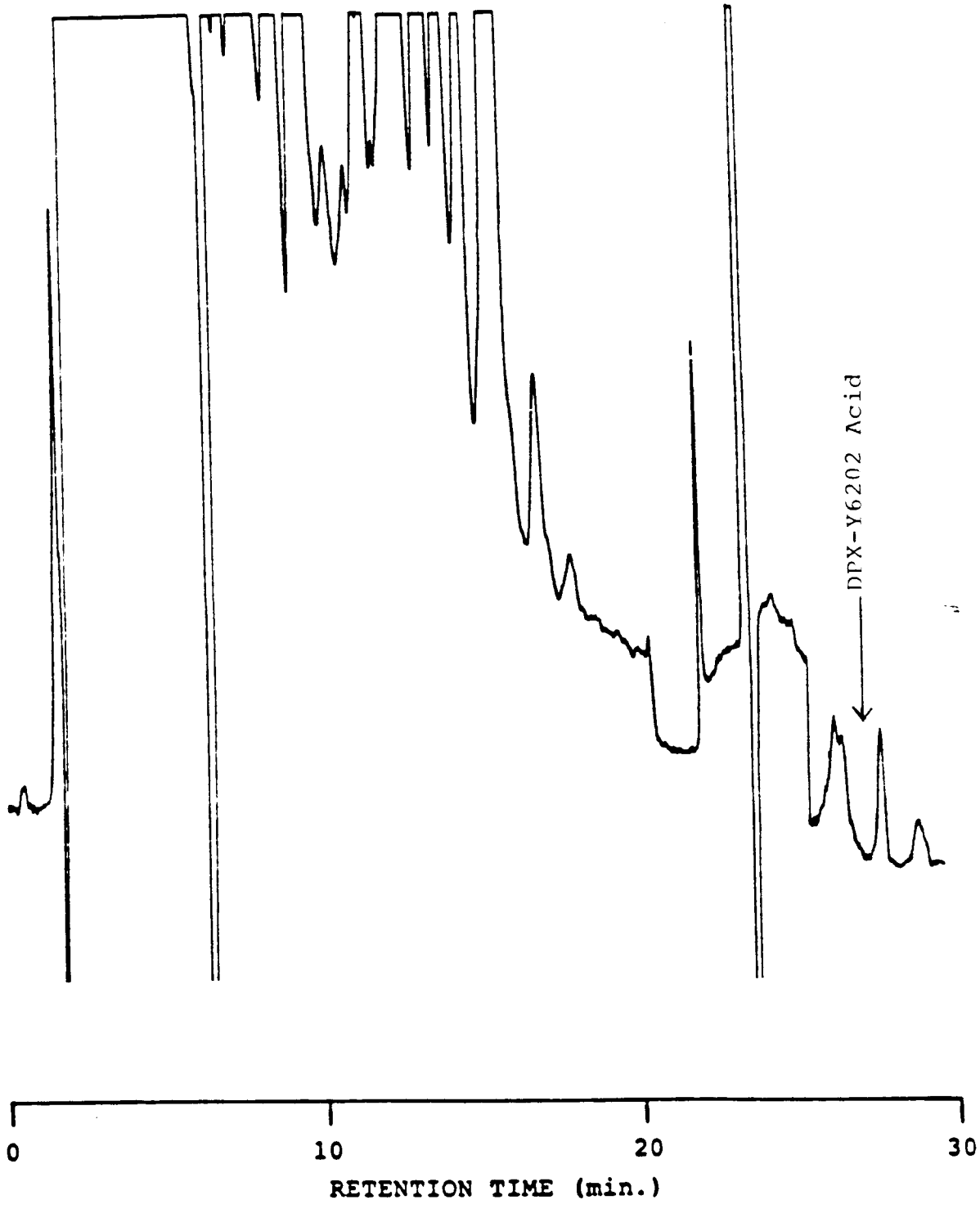


FIGURE 12: Chromatogram of a control chicken liver sample.

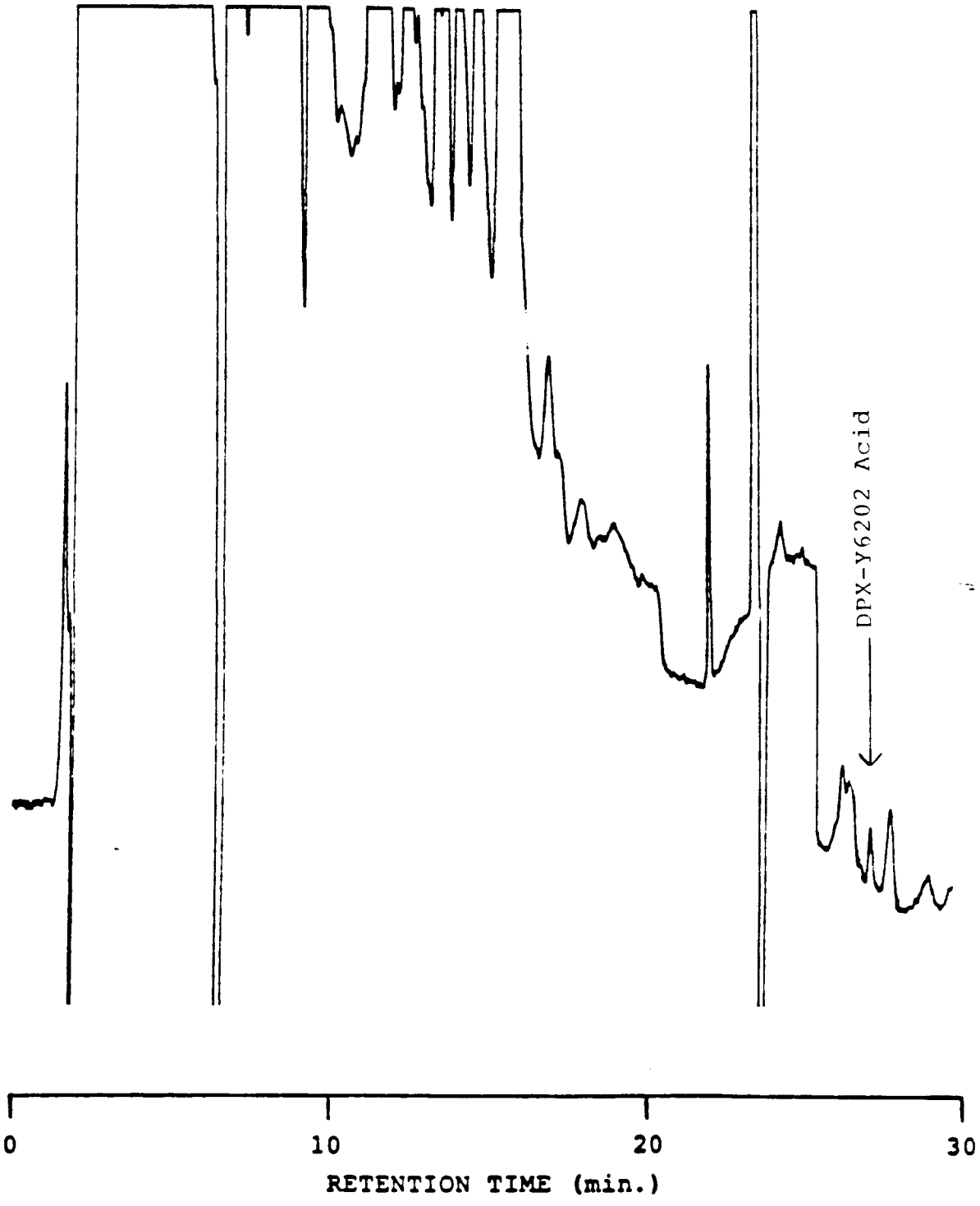


FIGURE 13: Chromatogram of the same control chicken liver sample as in Figure 12 fortified with 0.05 ppm DPX-Y6202 (Recovery = 80%).

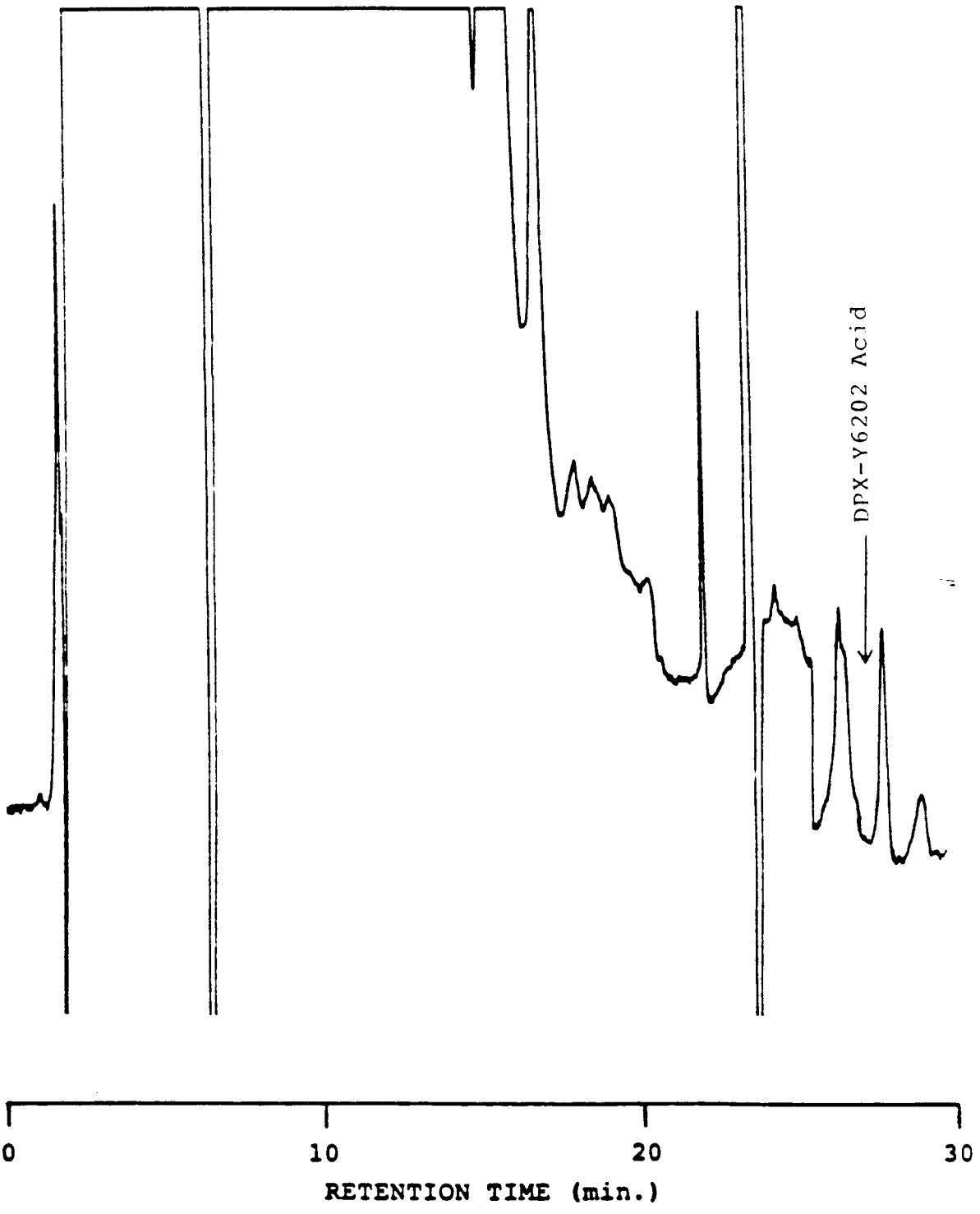


FIGURE 14: Chromatogram of a chicken liver sample from rep. II group fed 5.0 ppm DPX-Y6202.

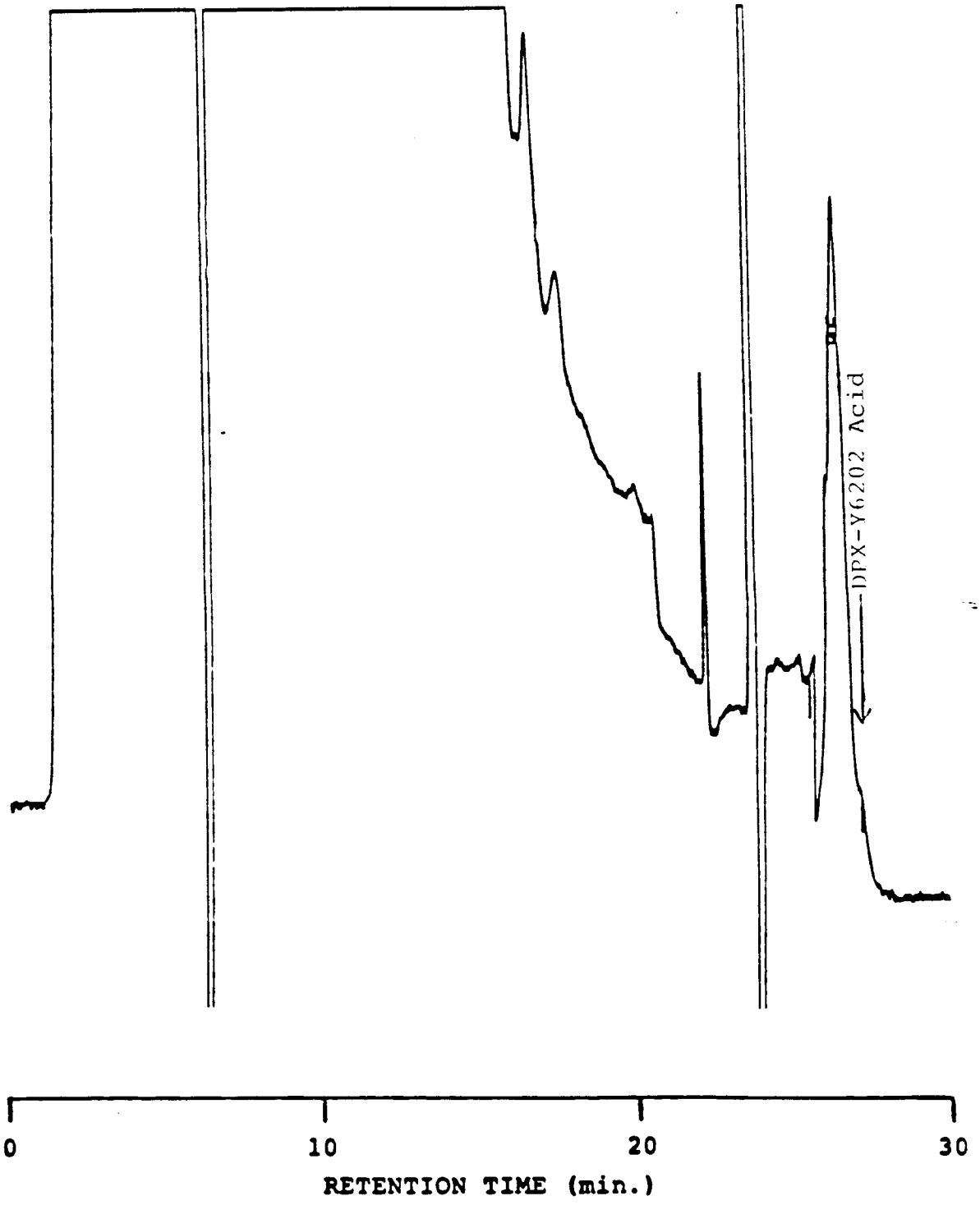


FIGURE 15: Chromatogram of a control chicken kidney sample.

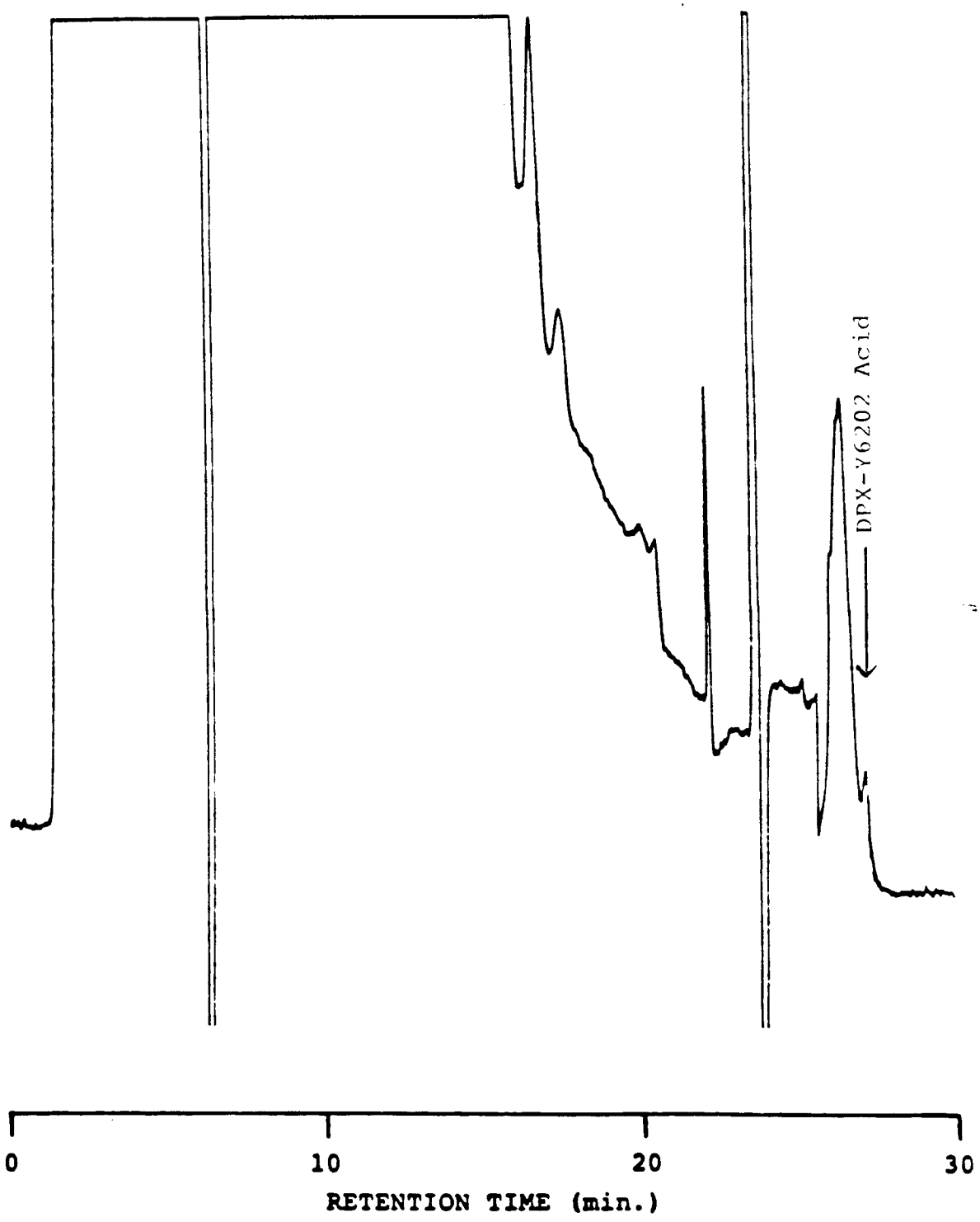


FIGURE 16: Chromatogram of the same control chicken kidney sample as in Figure 15 fortified with 0.05 ppm DPX-Y6202 (Recovery = 86%).

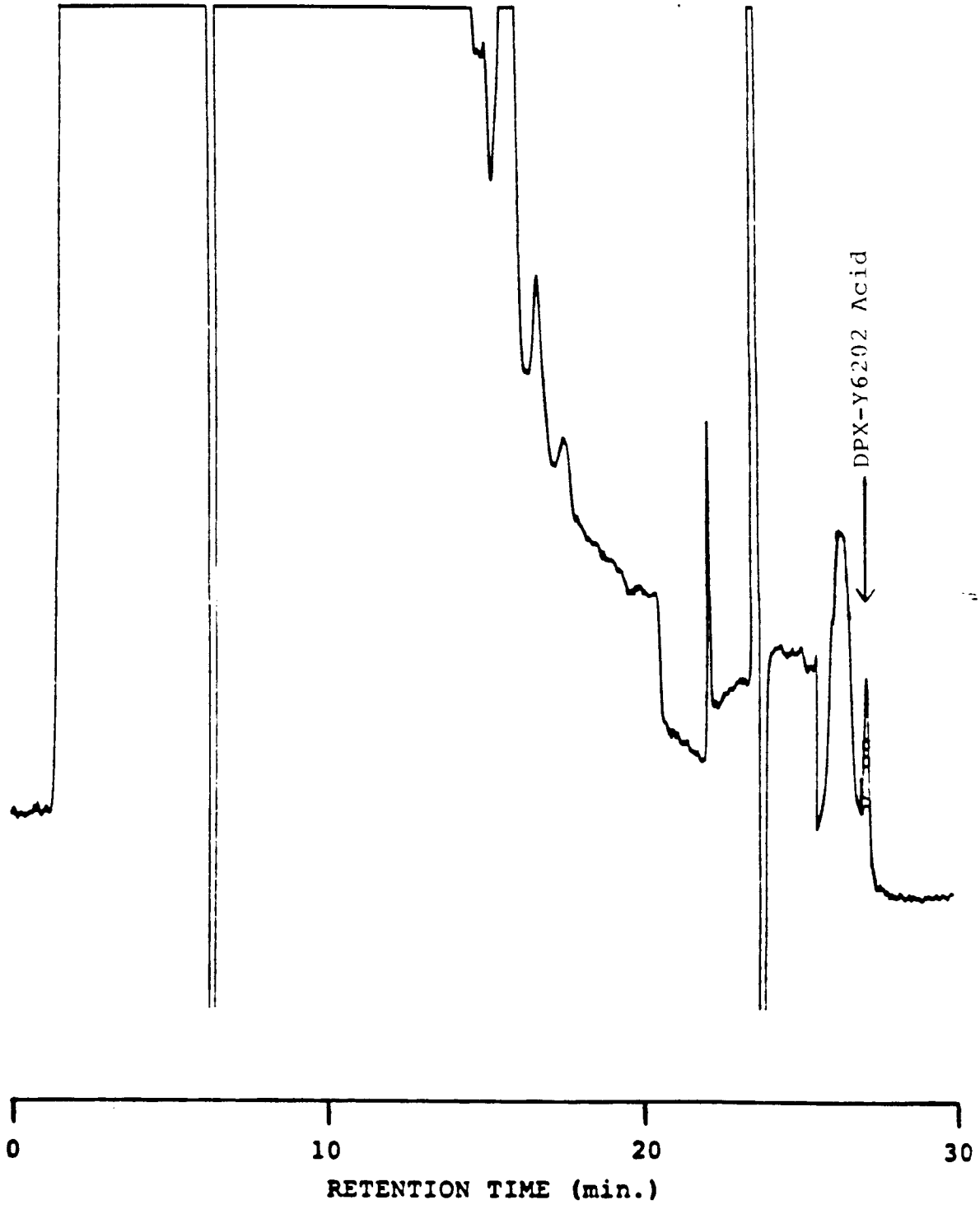


FIGURE 17: Chromatogram of a chicken kidney sample from rep. II group fed 5.0 ppm DPX-Y6202 (DPX-Y6202 Acid = 0.09 ppm).

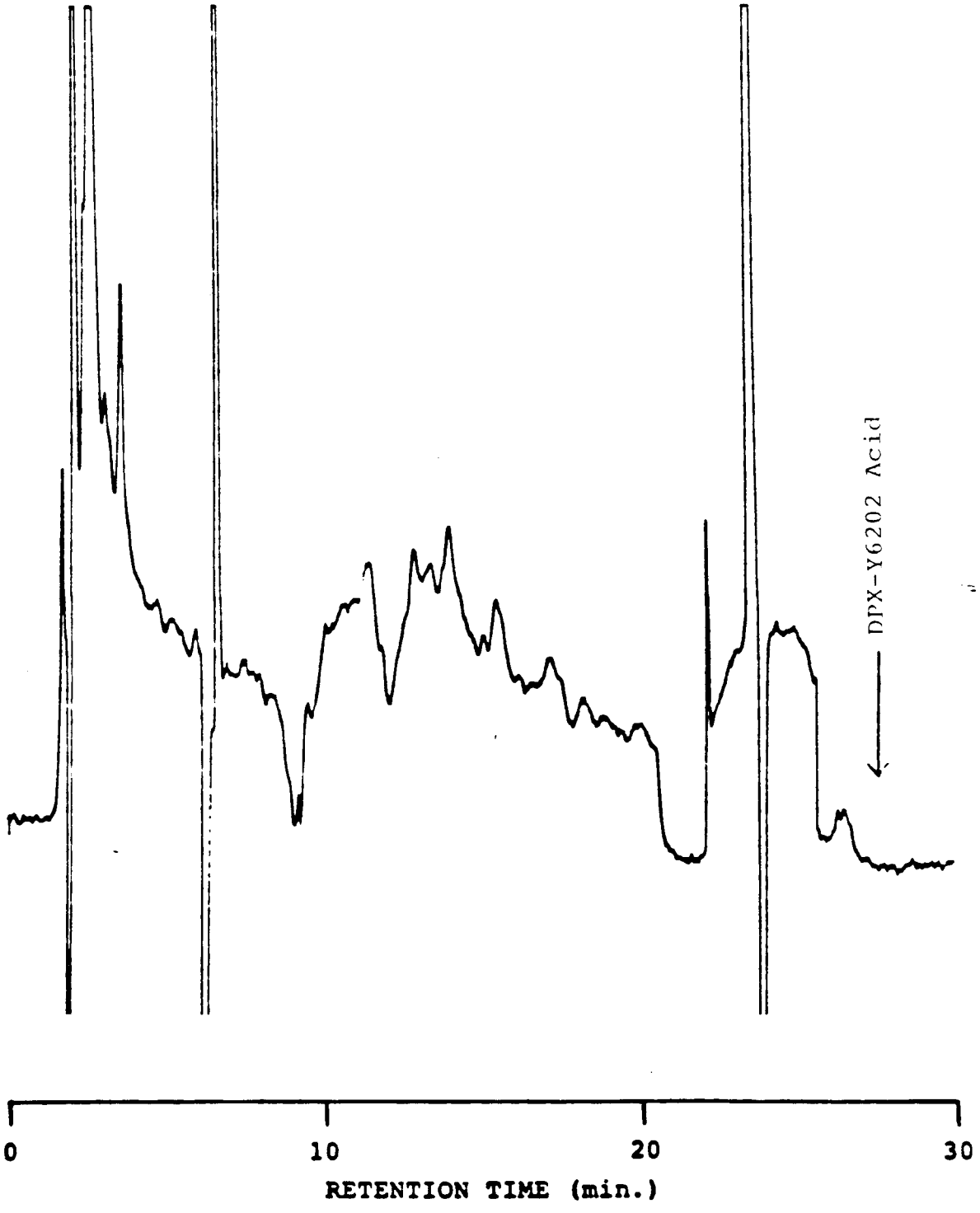


FIGURE 18: Chromatogram of a control chicken breast muscle sample.

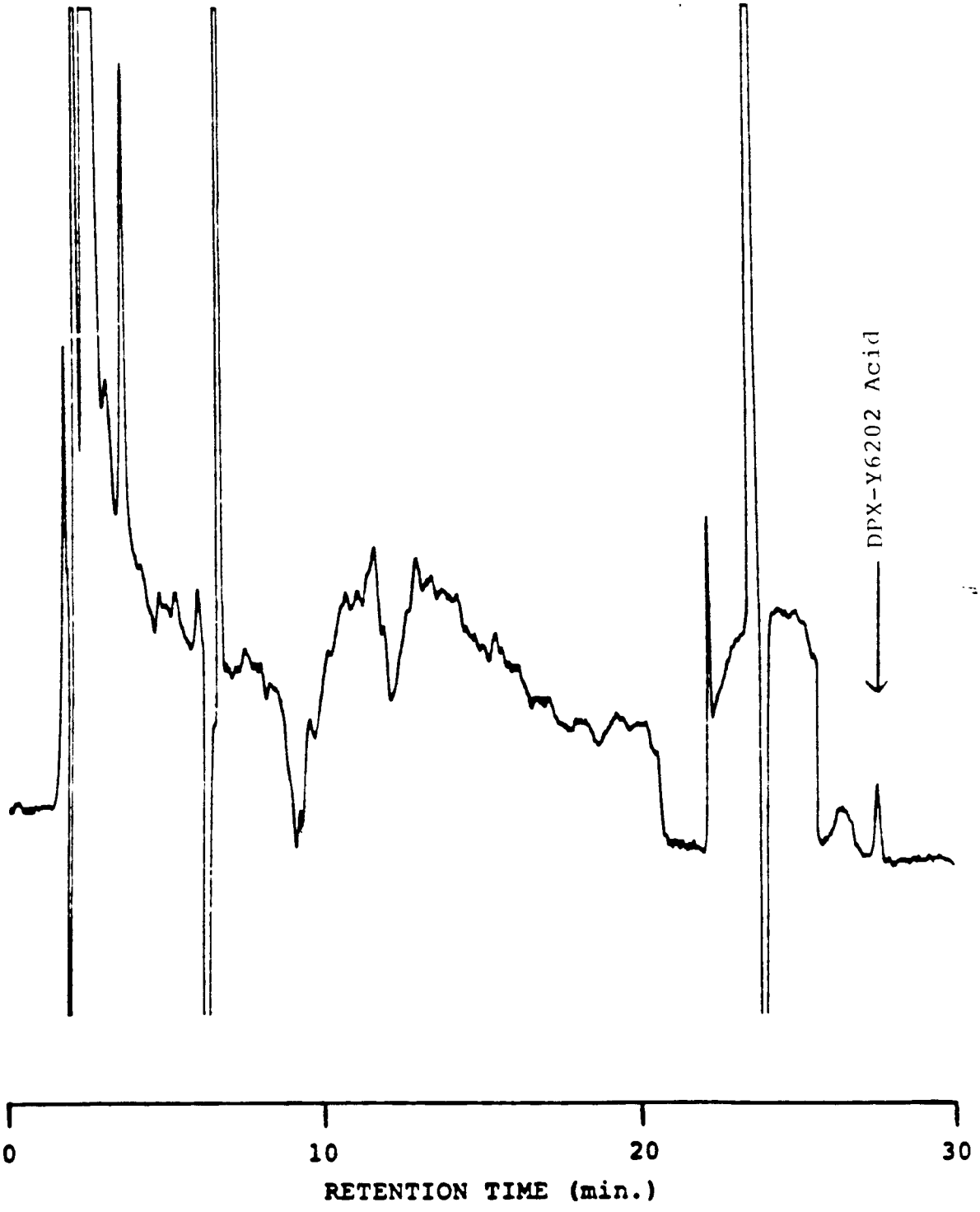


FIGURE 19: Chromatogram of the same control chicken breast muscle sample as in Figure 18 fortified with 0.02 ppm DPX-Y6202 (Recovery = 90%).

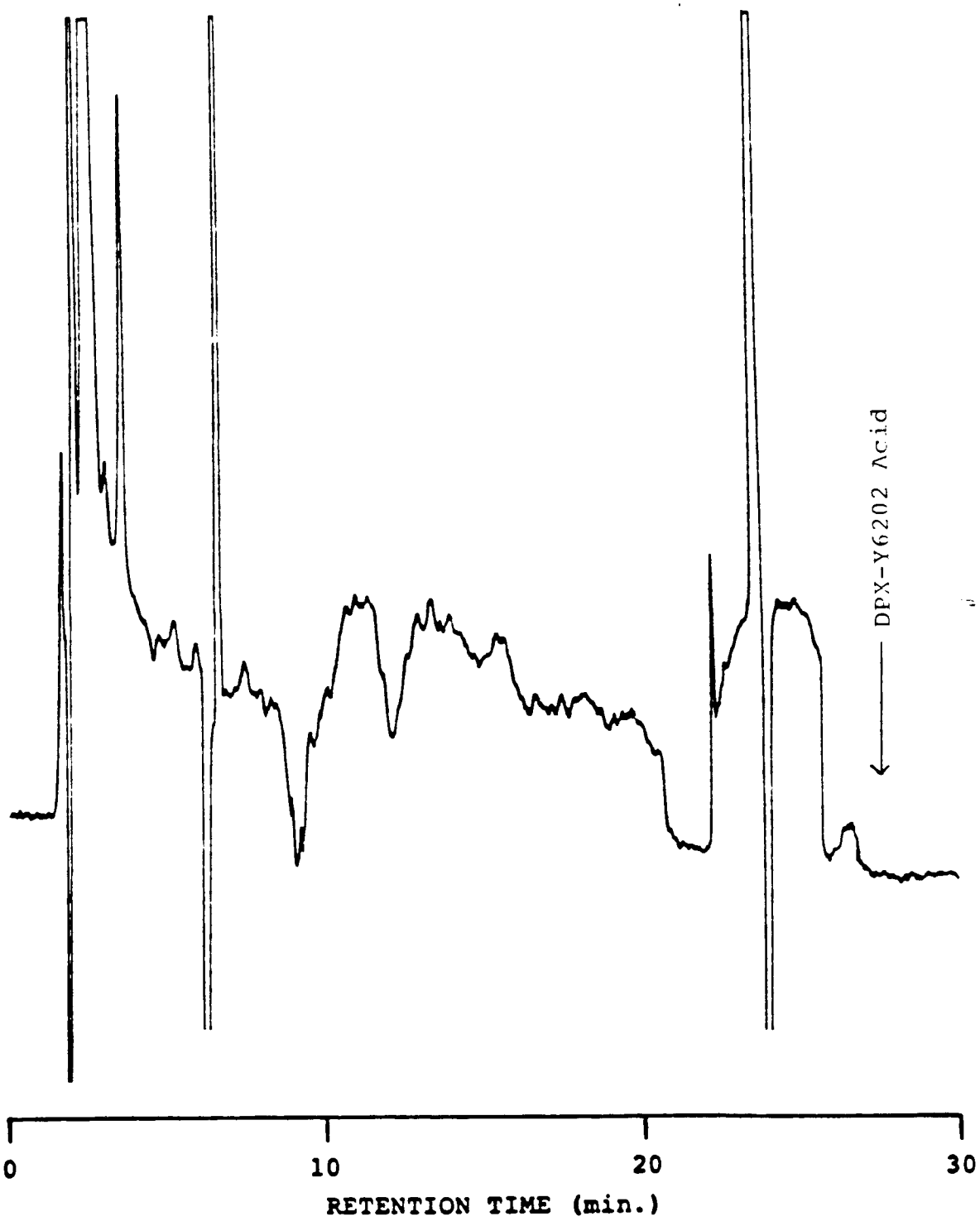


FIGURE 20: Chromatogram of a chicken breast muscle sample from rep. III group fed 5.0 ppm DPX-Y6202.

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