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DETERMINATION OF RESIDUES OF METSULFURON METHYL IN CROPS
BY LIQUID CHROMATOGRAPHY

By

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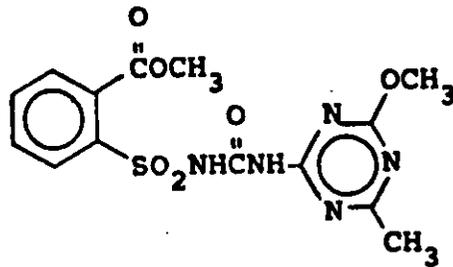
DETERMINATION OF RESIDUES OF METSULFURON METHYL BY
LIQUID CHROMATOGRAPHY

ABSTRACT

A method has been developed for determination of residues of metsulfuron methyl in grain, straw, and green forage samples. The method is based on its extraction from the crops with an acetone-aqueous buffer mixture and cleanup using a methylene chloride wash of a basic aqueous solution. This is followed by extraction into toluene from an acidic aqueous solution and further cleanup on a silica Bond Elut[®] cartridge. Final determination is by normal phase liquid chromatography using a photo-conductivity detector. The recoveries for 52 spiked samples averaged 84%. The lower level of quantitation for all crops was 20 ppb except for straw which was 50 ppb.

INTRODUCTION

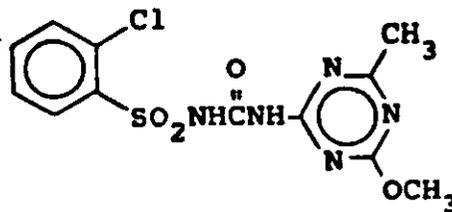
Benzoic acid, 2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-aminocarbonyl]aminosulfonyl]-, methyl ester (metsulfuron methyl) with the structure



Metsulfuron methyl

is a Du Pont experimental sulfonylurea herbicide. Metsulfuron methyl, formerly known as DPX-T6376, is effective in controlling a variety of weeds in cereal grain fields.

A method has been developed for determination of residues of metsulfuron methyl in various cereal grain crops and possible rotation crops. The method developed is similar in approach to that reported by Zahnow (1, 2) and Slates (3) for analysis of chlorsulfuron which is a similar sulfonylurea herbicide with the structure shown below.



Chlorsulfuron

After extraction and cleanup of the samples, metsulfuron methyl is determined by HPLC using a photo-conductivity detector. As little as 250 pg can be injected into the HPLC and measured. This corresponds to a detection limit of 20 ppb for all substrates except straw for which it is 50 ppb.

EQUIPMENT AND REAGENTS

A Du Pont model 8800 liquid chromatograph equipped with a column compartment, Valco valve, and a data system was used. The column was a Zorbax[®] SIL, 4.6 mm i.d. x 25 cm. Both the column and liquid chromatograph were purchased from Analytical Instruments Division, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware. The Tracor model 965 photo-conductivity detector used was purchased from Tracor Instruments, Austin, Texas.

For homogenization and extraction of samples, a Tekmar Tissumizer[®], model SDT-1810, with a model SDT-182 EN shaft and generator was employed. The Tissumizer[®] was purchased from Tekmar Company, Cincinnati, Ohio. A Vortex-Genie[®] mixer, purchased from Fisher Scientific, Pittsburgh, Pennsylvania, was used for mixing of samples in centrifuge tubes.

A Millipore[®] all glass filter apparatus, No. XX15 04700, with a 0.5 μ m Teflon[®] filter, No. FHUP 047 00, was used for filtering of mobile phase. Final cleanup of the samples was on silica Bond Elut[®] cartridges. Each cartridge contained 500 mg of sorbent packed in a 2.8 mL column. The cartridges were fitted with 75 mL reservoirs. A Vac Elut[®] vacuum manifold was used to pull solvents through the cartridges. The cartridges, reservoirs, and manifold were all purchased from Analytichem International, Harbor City, California. The Millipore[®] equipment was obtained from Millipore Corporation, Medford, Massachusetts.

For concentration of samples, a vacuum rotary evaporator with a 45°C water bath and pear-shaped flasks, No. K-608700, purchased from Kontes, Vineland, New Jersey were used. An N-EVAP[®], Organomation Assoc., Worcester, Massachusetts (water bath set at room temperature) concentrated the samples to final dryness under nitrogen. The Guth Universal[®] wash bottle was purchased from Fisher Scientific Company, Pittsburgh, Pennsylvania.

The reference standard of metsulfuron methyl was obtained from the Agricultural Chemicals Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware 19898. Brockman Activity I basic alumina was used to dry the mobile phase. The alumina, No. A-941, was purchased from Fisher Scientific. The HPLC grade solvents were obtained from J. T. Baker Chemical Co., Phillipsburg, New Jersey or Burdick and Jackson Laboratories, Muskegon, Michigan. All other chemicals were ACS reagent grade and were obtained from Fisher Scientific.

PROCEDURE

Glassware Deactivation

All glassware used during the procedure was silanized to deactivate it. The silanizing reagent was prepared by mixing 30 mL of dichlorodimethyl silane and 30 mL of chlorotrimethyl silane with 1.0 liter of toluene. The glassware was dipped in the silanizing reagent for 2 minutes and then rinsed with toluene, methanol, distilled water, and methanol respectively. After drying, the glassware was ready for use. The glassware needed to be resilanized after being used for six or seven sets of samples.

Isolation

A representative 25 gram sample (10 grams for straw) was weighed into a 250 mL glass centrifuge bottle and 80 mL of extraction solvent added (170 mL for straw). The extraction solvent was made by mixing 80 mL of acetone with 20 mL of buffer B, made by adding 0.82 grams of sodium acetate and 0.5 mL of glacial acetic acid to 1.0 liter of distilled water.

A set of samples was sequentially homogenized with the Tekmar Tissumizer[®] for 1.0 minute each. Between each sample the Tissumizer[®] probe was rinsed with some extraction solvent from a Guth[®] wash bottle and the rinse added to the sample

bottle. The samples were then centrifuged at 2500 rpm for 5 minutes and the liquid decanted through a glass wool plug in a funnel and collected in a 250 mL volumetric flask. After the first extraction of the sample set, the Tissumizer[®] probe was disassembled and cleaned with hot water, distilled water, and acetone. Before using again, it was run in a centrifuge bottle containing 80 mL of extraction solvent to prevent cross-contamination.

The extraction and centrifugation steps described above were repeated twice more with 70 mL of the extraction solvent each time (once with 100 mL for straw). After all extractions had been combined in the volumetric flask, the samples were made to volume with the extraction solvent.

Cleanup

A 25 mL aliquot of each sample was pipetted into a 250 mL separatory funnel which contained 100 mL of Buffer A, made by dissolving 16.8 grams of sodium bicarbonate and 21.1 grams of sodium carbonate in 2.0 liters of distilled water. The samples were then washed with 3 x 50 mL of methylene chloride by shaking for 1.0 minute each time. The bottom methylene chloride phase was removed each time and discarded. After the third wash, the aqueous phase was transferred to a 250 mL beaker. The pH was

adjusted to 3.5 using a pH meter and 10% hydrochloric acid. Once the samples had been acidified, the next extraction step was carried out immediately.

The acidified samples were transferred back to the 250 mL separatory funnels and 50 mL of toluene added to each beaker. After swirling, the toluene was added to the separatory funnel. The samples were shaken for 1.0 minute, the bottom aqueous layer was transferred to a 250 mL beaker, and the toluene then drained into a 250 mL glass centrifuge bottle. The aqueous solution in the beaker was poured back into the separatory funnel and extracted twice more with 50 mL of toluene each time and these extracts also added to the centrifuge bottle. After the third extraction, about 10 mL of the aqueous phase was also added to the centrifuge bottle and the samples centrifuged for 5 minutes. A silica Bond Elut[®] cartridge for each sample, fitted with a 75 mL reservoir, was first rinsed with 10 mL of 2-propanol and then 10 mL of toluene. The top toluene layer was removed from each centrifuged sample with a 50 mL glass syringe and passed through a prerinsed silica Bond Elut[®] cartridge (the metsulfuron methyl adsorbed on the silica). Care was taken to avoid removing any buffer with the syringe. To each centrifuge bottle was added 25 mL more of toluene and the samples were centrifuged again. The toluene layer was removed as before and also passed through the silica Bond Elut[®] cartridge. The silica cartridge was then rinsed with an additional 10 mL of

toluene and 1/2 mL of HPLC mobile phase (see the chromatographic section). The reservoir was removed from the cartridge and the cartridge removed from the vacuum manifold.

To elute the metsulfuron methyl from the silica Bond Elut[®] cartridge, a 13 mL glass-stoppered centrifuge tube was placed in a 250 mL filter flask. The flask was attached to a vacuum source at the side arm. A 4 inch, 16 guage luer hub stainless steel needle was inserted through a No. 6 rubber stopper. The stopper was placed on the filter flask with the needle extending into the centrifuge tube. The silica Bond Elut[®] cartridge was fitted to the luer hub of the needle. To elute the metsulfuron methyl, HPLC mobile phase (5.0 mL) was pulled by vacuum through the cartridge and collected in the centrifuge tube. The samples were then concentrated to dryness with the N-Evap[®] and stored at <4°C until analyzed.

For HPLC analysis, each sample was then redissolved in 2.0 mL of HPLC mobile phase (see chromatographic section), ultrasonically mixed for a minute and vortex mixed. Each sample was then filtered through a Millipore[®] Millex[®]-SR filter into a clean 13 mL glass-stoppered centrifuge tube.

Liquid Chromatography

The liquid chromatograph used was a Du Pont Model 8800 with a Du Pont data system. A Tracor Model 965 photo-conductivity detector equipped with a mercury lamp was used because of its sensitivity and selectivity for metsulfuron methyl. The ion exchange resin tube and pump were removed from the detector because they were not needed. The detector was further modified by placing a Nupro metering valve (Model SS-25A-TFE) in the exit line from the reference conductivity cell. This was then adjusted to equalize the flow through the reference and analytical conductivity cells.

The HPLC mobile phase was made by mixing 690 mL of cyclohexane, 195 mL of 2-propanol, and 115 mL of methanol in a 2.0 liter beaker. Fifty grams of Brockman Activity I basic alumina was added and the solution stirred for 10 minutes. The alumina was allowed to settle and the mobile phase filtered with a Millipore[®] all glass filter apparatus using a Teflon[®] filter. To the filtered solution was added 3.0 mL of glacial acetic acid and 100 μ L of deionized water and the solution stirred for 10 minutes. (see Appendix I for an alternate mobile phase)

A column conditioning solution was made by mixing together 400 mL of 2-propanol, 400 mL of methanol, 200 mL of acetic acid, and 40 mL of distilled-deionized water. This solution was also filtered with the filter apparatus and Teflon[®] filter.

The Zorbax[®] SIL, 4.6 mm i.d. x 25 cm, column was conditioned with the conditioning solution before being used. The solution was pumped at 0.70 mL for four hours and then the column equilibrated with mobile phase for three hours at the same flow rate. The conditioning procedure was repeated whenever necessary to restore columns which lost efficiency from contamination.

For analysis of samples, an injection volume of 10 μ L was used and the oven was operated at 35°C. The flow rate was set at 1.0 mL/minute. The detector attenuation was operated on 1 x 1 and any attenuation of the detector signal was by the data system.

A 100 μ g/mL stock solution was made by dissolving 10 mg of metsulfuron methyl in 100 mL of ethyl acetate. Dilutions were made from the stock at 0.5, 1.0, and 2.5 μ g/mL by pipetting 0.5, 1.0, and 2.5 mL of the stock into 100 mL volumetric flasks and making to volume with ethyl acetate. These standards were used in fortifying control samples for determination of recoveries.

A 1.0 μ g/mL standard in HPLC mobile phase was made by pipetting 1.0 mL of the 100 μ g/mL stock standard into a 100 mL volumetric flask. The ethyl acetate was removed with dry nitrogen and the volumetric flask made to volume with HPLC mobile phase. Working HPLC standards (see Table 1) from this 1 μ g/mL solution were prepared weekly by using a syringe to pipette the standard into 5 or 10 mL volumetric flasks and by making to volume with HPLC mobile phase. The HPLC standards were injected on the HPLC interspersed with the samples being analyzed.

Calculations

The sensitivity for each standard S , in (mm-mL)/ng units, was calculated by the equation,

$$S = \left(\frac{P_S}{C_S} \right) (A) \quad (1)$$

where P_S was the peak height in millimeters. C_S was the concentration of the standard in ng/mL, and A was the attenuation. The average sensitivity, S_a , was calculated and used for calculation of sample concentrations.

The sample concentration, C , in ng/g units (ppb) was calculated using the equation:

$$C = \left[\left(\frac{P}{S_a} \right) (V) (D) \right] \left(\frac{1}{W} \right) \quad (2)$$

where P was the sample peak height in millimeters, V was the final sample volume in mL, D was the sample aliquot factor which was equal to 10, and W was the weight of sample used in grams.

RESULTS AND DISCUSSION

Recoveries for several cereal grain and rotation crops are summarized in Table 2. The average recovery for the 52 spiked samples was 84%. A wheat forage control and the same control spiked at the detection limit of 20 ppb (100% recovery) are shown in Figure 1. A wheat straw control and the same control spiked at the detection limit of 50 ppb (84% recovery) are shown in Figure 2.

Early work with 25 gram samples carried through the solvent extraction steps indicated serious emulsion problems with the more oily crops. Early work also indicated serious problems with removing the acetone from the Buffer B after the initial extraction of the crops. When stripped on a rotary evaporator, the samples foamed badly.

To eliminate these problems, it was decided to use an aliquot of the sample extract equivalent to a sample weight of 2.5 grams. Reducing the amount of crop co-extractables eliminated the emulsion problems. The small amount of acetone (20 mL) in the aliquot was removed from the basic aqueous solution by the methylene chloride during the wash step. Therefore, it did not interfere during the rest of the cleanup steps.

Aliquoting of the sample was made possible by the sensitivity of the photo-conductivity detector to metsulfuron methyl. The detector was sensitive to 250 pg of metsulfuron methyl in a 10 μ L injection (25 ng/mL). For an aliquot factor

of one tenth and a final sample volume of 2.0 mL, a detection limit of 20 ppb was obtained for a 25 gram sample and 50 ppb for a 10 gram straw sample.

A 10 gram sample of straw was used because that is all that would fit into the 250 mL centrifuge bottle used for homogenization of the sample. The bulkiness of the straw also required larger volumes of solvent than did other crops for successful homogenization with the Tekmar homogenizer. To keep the total solvent volume below 250 mL, it was necessary to use only two extractions for straw rather than the three used for other crops. This had little effect on the final recovery since most of the metsulfuron methyl was extracted in the first two extractions.

The alternate mobile phase described in Appendix I was developed for high sensitivity work because the baseline is more stable at the elution point for metsulfuron methyl. This mobile phase reduces the occurrence of negative dips in the baseline which can occur near the retention time for metsulfuron methyl. Although all the recoveries used in this method were obtained with the methanol based mobile phase, I would recommend that the alternate mobile phase be used since it gives a better chromatographic baseline.

REFERENCES

- 1) Zahnow, E. W.: "Analysis of the Herbicide Chlorsulfuron in Soil by Liquid Chromatography". J. Agric. and Food Chem. 1982, 30, 854.
- 2) Zahnow, E. W.: "Analysis of Chlorsulfuron and Metabolite A", AMR-70-82, Agricultural Chemicals Department, Research Division, E. I. du Pont de Nemours and Co., Wilmington, Delaware.
- 3) Slates, R. V.: "Determination of Chlorsulfuron Residues in Grain, Straw, and Green Plants of Cereals by High Performance Liquid Chromatography." J. Agric. and Food Chem. 1983, 31, 113.

TABLE 1

HPLC Standards Preparation

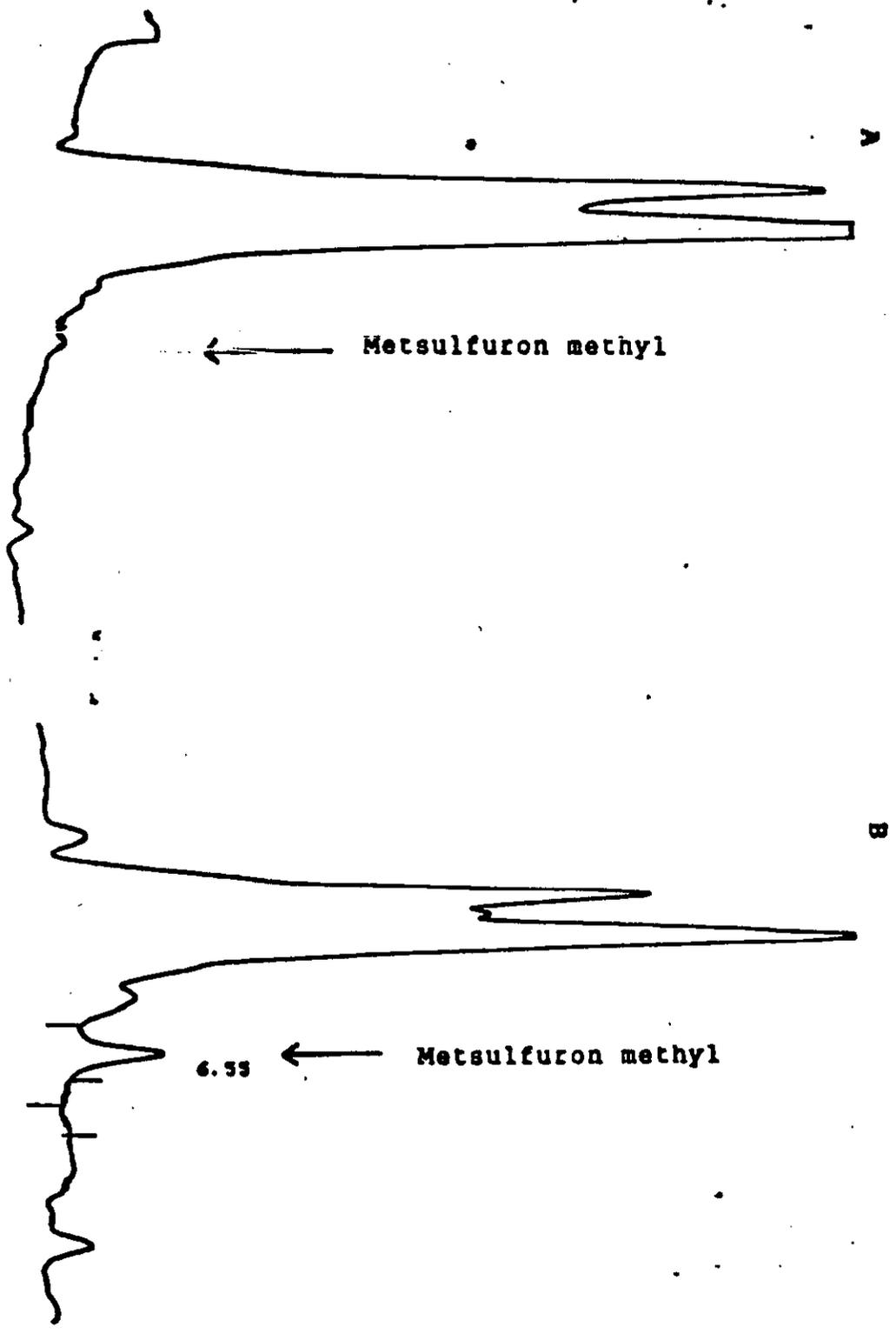
<u>Standard Concentration (ng/mL)</u>	<u>Volume of 1.0 µg/mL Standard Used (mL)</u>	<u>Final Volume (mL)</u>
250	2500	10
200	1000	5
150	750	5
100	1000	10
50	500	10
25	250	10

TABLE 2**Recovery Data**

<u>Substrate</u>	<u>Number of Recoveries</u>	<u>Spiking Range (ppb)</u>	<u>Recovery Range (Percent)</u>	<u>Average Recovery (Percent)</u>
Barley (Grain)	5	20 - 100	80 - 89	85
Corn (Green Forage)	2	20 - 100	72 - 95	84
Kidney Bean (Green Forage)	2	20 - 40	75 - 80	78
Rapeseed	5	20 - 100	78 - 90	85
Soybeans (Grain)	5	20 - 100	80 - 93	86
Wheat (Grain)	13	20 - 100	66 - 95	82
Wheat (Green Forage)	9	20 - 100	78 - 110	93
Wheat (Straw)	11	50 - 250	60 - 92	81

Chromatograms of a) a wheat forage control and b) the same control spiked at 20 ppb with metsulfuron methyl

FIGURE 1



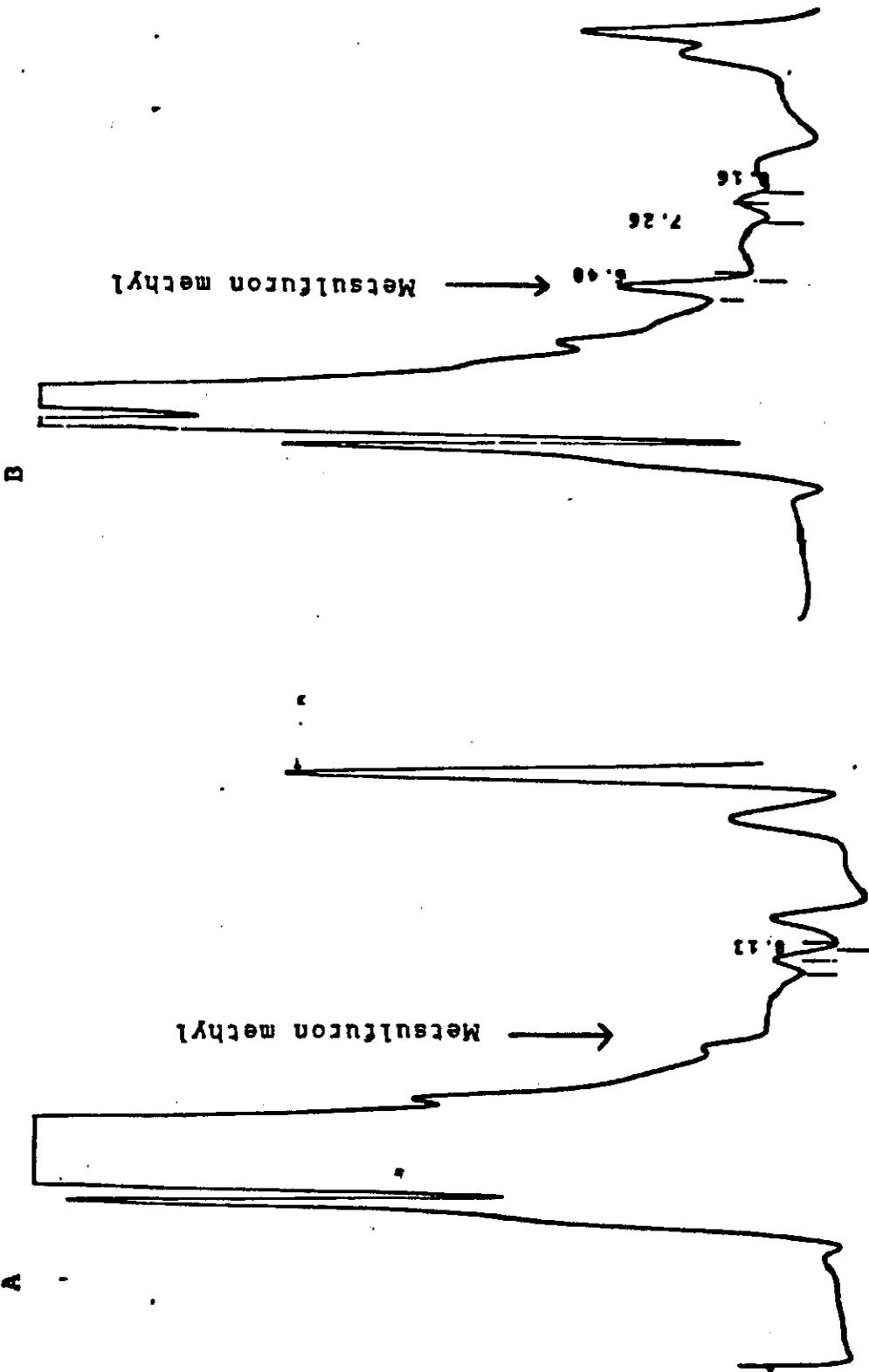


FIGURE 2

Chromatograms of a) a wheat straw control and b) the same control spiked at 50 ppb with metsulfuron methyl

Appendix I

Alternate Mobile Phase

The alternate mobile phase was made by mixing 750 mL of cyclohexane, 175 mL of 2-propanol, and 110 mL of acetonitrile together in a beaker. When these three components were mixed, the temperature was lowered and they would not mix completely. Therefore, the solution was either warmed by sitting in a pan of warm water or else it was stirred until it warmed enough for all three components to mix. After mixing well, the solution was filtered with a Millipore® all glass filter apparatus using a 0.45 µm Teflon® filter. After filtering, 1.0 mL of glacial acetic acid and 100 µL of deionized H₂O was added and the solution stirred for 10 minutes.

Solution B to dissolve standards and samples was made the same as the mobile phase except that the acid and water were not added to the solution. The flow rate for this mobile phase was 0.5 mL/min.