PLANT UPTAKE OF FLUOROBENZOATES USED AS SOIL AND GROUNDWATER TRACERS

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ABSTRACT

Fluorobenzoates are being widely used as conservative tracers for soil and groundwater studies. Several studies conducted recently prove their usefulness as good soil and groundwater tracers. To use these compounds in agronomic situations, a systematic study needed to be done on the plant uptake and toxicity of these compounds. Green house experiments were conducted to study the plant uptake and toxicity of three representative fluorobenzoates namely, 2,6-DFBA, 3,4-DFBA and PFBA. The well established conservative tracer bromide was used as a control for this study. Alfalfa, barley and canola plants were selected for the study. Tracers were applied to each plant at a concentration of 50 mg/L soil solution. The plants were tested separately for the uptake of the three tracers. An analytical method was developed for the analysis of fluorobenzoates in plant material. Plant extracts and soil extracts were analyzed using HPLC in order to determine a mass balance for the added tracers. Analysis of alfalfa, barley and, canola soil extracts resulted in the recovery of 72%, 69%, 51% of the applied PFBA, 83%, 59%, 30% of the applied 2,6-DFBA and 39%, 42%, 34% of the applied 3,4-DFBA, respectively. The analytical results of alfalfa, barley and canola plant extracts indicate an average uptake of 9%, 22%, 49% of the applied 2,6-DFBA, and 0.1%, 2%, 19% of the applied PFBA, respectively. An average mass balance of 84% and 70% was achieved for the 2,6-DFBA and PFBA treatments respectively. Metabolism within the plant material is suspected to be the reason for the missing mass balance.

ABBREVIATIONS

2,6-DFBA - 2,6-difluorobenzoic acid; 3,4-DFBA - 3,4-difluorobenzoic acid; PFBA - pentafluorobenzoic acid; *O*-TFMBA - *ortho*-trifluoromethyl benzoic acid; *m*-TFMBA - *meta* trifluoromethylbenzoic acid; 3,5-DFBA - 3,5-difluorobenzoic acid; TFBA - trifluorobenzoic acid; TEFBA - tetrafluorobenzoic acid; CPM - counts per minute; HPLC - high performance liquid chromatography; GC - gas chromatography; UV - ultraviolet; NMSU - New Mexico State University; pK_a - negative log of acid dissociation constant; K_{ow}- octanol-water partition coefficient. Sample names or numbers - all the sample names or numbers are abbreviated with a one letter followed by three numbers. The first letter stands for the crop type (A - alfalfa, B - barley, C - canola) and the first number stands for the repetition. The other two numbers does not have any significance.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

A groundwater tracer is any physical or chemical signal that can be carried by water and thus throws some light on the character of porous media through which the water flows. Groundwater tracers are essentially used to study the various hydrological properties of soils and aquifers such as water flow direction and velocity, water flux, solute dispersion, solute sorption and retardation, hydraulic conductivity, porosity, dispersivity and other hydrological parameters. A groundwater tracer can be either naturally occurring, such as a geothermal plume or stable isotopes, or it can be injected anthropogenically, such as dyes and other chemical compounds. An excellent review of several types of groundwater tracers and their usefulness was done by Davis et.al. (1980).

Even though several types of tracers exist, anthropogenically added chemical compounds such as anions and dyes have gotten most of the attention either due to their low adsorption to porous media or due to their ease of detection. For a chemical compound to be a good groundwater tracer it should meet certain requirements such as low natural abundance; minimum or no interaction, either physical or chemical, in the porous medium or soil; chemical and biological stability for a considerable length of time; no alteration of the natural flow direction of water; environmental acceptability; and easy and economical analysis (Davis et. al., 1980; Bowman, 1984b).

In addition to the above requirements, a tracer to be used in soil water studies, especially under agronomic conditions, should meet some additional requirements.

The greater surface activity and solid/water ratios of soils result in higher sorption, due to which many compounds used as groundwater tracers may not be useful for soil water studies (Bowman, 1984b). In addition to this, the most important requirement is nontoxicity to the plants if used in agricultural situations. Preferably, the tracer should not be taken up by plants and it should not have any deleterious effect on plant growth, maturity or yield.

Although no ideal groundwater tracer exists, deuterated and tritiated water, and low molecular weight anions such as chloride, bromide and nitrate approach the behavior of an ideal groundwater tracer. However these compounds have certain limitations such as high natural abundance (chloride, often greater than 100 mg/L), lack of stability (nitrate), high costs (deuterated water), or radioactivity (tritiated water). Bromide, due to its usual low background concentrations, minimum interaction with soils, and ease of quantitative analysis, is used most commonly as a groundwater tracer (Bowman, 1984b). Even though bromide approaches close to ideality as a groundwater tracer, recent field studies by Kung (1990) have indicated that up to 55% of the bromide applied as a tracer was taken up by potato plants, and 44% was reintroduced into the soil after the death and decay of the plants. This kind of uptake of soil water tracer and reintroduction into the soil after decay is a serious disadvantage for the interpretation of solute transport studies. Laboratory studies on bromide uptake by Gish and Jury (1982) indicated that 2% of applied bromide can be taken up by wheat. Owens et.al. (1985) reported about 30% uptake of bromide by grass in a field study.

Recent studies indicate that a suite of difluorobenzoate isomers and PFBA have properties that are suitable for good groundwater tracers. These compounds, due to their low pK_as (<4.0), exist as anions at neutral to basic pH values, are chemically and microbially stable, and can be easily analyzed at μg/L levels using HPLC (Bowman & Gibbens, 1992). Table 1 shows several fluorobenzoates which were either used or have potential to be used as groundwater tracers. Several studies done recently, both in the field and in laboratory columns, have proven the usefulness of fluorobenzoates as good soil and groundwater tracers. Transport of the fluorobenzoates was similar to that of bromide in these studies (see Previous Work Section). However, none of these studies was conducted in the presence of growing plants.

Fluorobenzoates can be used as tracers when any of the other anions cannot be used or if an additional number of tracers are required. But to use fluorobenzoates as tracers in agronomic situations, where there is a high possibility of exposure to plants, a systematic study needed to be done on the plant toxicity and plant uptake of these compounds.

This work presents the results of plant uptake of fluorobenzoates, part of a major project entitled " Plant Toxicity and Plant Uptake of Fluorobenzoates Used as Soil and Groundwater Tracers". The project was funded by the United States Department of Agriculture and was done in cooperation with New Mexico State University, Las Cruces, New Mexico. The project was divided into three phases with three corresponding objectives. The first two phases were done by NMSU.

Table 1. Various fluorobenzoates that either have been used or can be used as groundwater tacers. (Benson & Bowman, 1994)

Compound	pK_a	Aqueous diffusion coefficient (m^2s^{-1} X 10^{-10})	Detection limit ng
2,3-DFBA	3.29	7.6	2.9
2,4-DFBA	3.58	7.6	NR*
2,5-DFBA	3.30	7.6	2.8
2,6-DFBA	2.85	7.6	2.1
3,4-DFBA	3.83	7.6	2.1
3,5-DFBA	3.59	7.6	2.6
2,3,4-TFBA	3.30	7.5	1.8
2,3,6-TFBA	2.82	7.5	3.6
2,4,5-TFBA	3.28	7.5	2.0
2,4,6-TFBA	2.83	7.5	NR
3,4,5-TFBA	3.54	7.5	2.1
2,3,4,5-TEFBA	3.08	7.4	2.5
2,3,5,6-TEFBA	2.71	7.4	4.2
PFBA	2.72	7.2	2.5

* not reported

The three phases of the project were-

1. Determine the levels of fluorobenzoates that inhibit germination of representative crop seeds.

2. Determine the levels of fluorobenzoates that inhibit growth of established representative crop plants.

3. Determine the degree of fluorobenzoate uptake by established representative crop plants.

In this study the results of the third phase are presented. Green house experiments were conducted to study the uptake of the three fluorobenzoates 2,6-DFBA; PFBA; and 3,4-DFBA by alfalfa, barley and canola plants. The fluorobenzoates used for this study were selected based on their proven usefulness and range of chemical characteristics (see Materials and Methods Section). Bromide, the well established conservative groundwater tracer, was used as a control for the uptake studies. Use of fluorobenzoates in the presence of plants would be warranted if they show less uptake than bromide and if they are nontoxic to plants.

PREVIOUS WORK

Bromide, which is widely accepted as a conservative groundwater tracer, was systematically studied for plant uptake by Kung (1990). In his field study using potato plants Kung showed that up to 55% of applied bromide was absorbed by plants. Gish and Jury (1982) in a column study showed that 2% of applied bromide can be taken up by wheat. Owens et. al. (1985) documented that 30% of applied bromide can be taken up by grass in a field study.

Fluorinated benzoic acids have been recently used as water tracers in a variety of soil and groundwater environments. Bowman and Gibbens (1992) evaluated the transport and degradation properties of several difluorobenzoate isomers relative to bromide and recommended that these compounds can be used as tracers based on their long term stability and conservativeness in porous media. They concluded that aromatic acids with direct ring substitution by fluorine have shown the greatest long term resistance to chemical and biological breakdown in the environment. Jaynes (1994) evaluated fluorobenzoates as tracers in fertile, high organic soils and concluded that 2,6-DFBA and PFBA were the most suitable, having transport properties similar to bromide and minimum retardation or degradation.

PFBA, 2,6-DFBA, *o*-TFMBA, and *m*-TFMBA were used to follow the downward movement of individual slugs of irrigation water in flood-irrigated agricultural fields in central Arizona (Bowman & Rice 1986a, 1986b). PFBA, 2,6-DFBA and *o*-TFMBA were used to determine surface origin points of subsurface discharge resulting from rainfall on a forested hillslope in east-central Maine

(Hornberger et al., 1990). In a large-scale multi-year aquifer tracer test in Mississippi, Young & Boggs (1990) showed that PFBA, 2,6-DFBA and *o*-TFMBA behaved essentially similar to bromide. Stensrud et. al (1990) used PFBA, *o*-TFMBA and *m*-TFMBA to characterize aquifer heterogeneity in highly fractured dolomite in southeastern New Mexico.

All the above studies indicated that, as a class of compounds, fluorobenzoates have desirable properties for water tracers in soil and groundwater. However, all these studies were conducted either on bare soils or in aquifers.

To use the fluorobenzoate tracers for solute transport studies in agricultural soils, plant uptake and plant toxicity of these compounds need to be studied. Several toxicity studies done on a variety of plants, using a wide range of substituted benzoic acids, especially phenolic acids (benzoic acids substituted with a phenol group), indicated that they were toxic to plants.

A wide variety of substituted benzoic acids (phenolic acids) exist naturally in plants and soils, either as products of plant degradation or of microbial generation. Several low molecular weight phenolic acids, particularly p-hydroxybenzoic, vanillic, p-coumaric, and ferulic acids occur widely in soils (Whitehead, 1964). Wang et. al. (1967) extracted and identified a number of benzoic acid derivatives from soils and showed that p-hydroxybenzoic acid inhibits plant growth in corn, soybean, wheat and sugar cane plants. Toussoun et. al. (1968) showed that 60% of the total phytotoxicity to tobacco seeds resulting from decomposing barley plant material was due to four aromatic acids, namely benzoic acid, phenylacetic acid, 3-phenyl propionic acid, and

4-phenyl-butyric acid. Benzoic acid and phenylacetic acid were the major components in the extract.

In a series of articles Glass (1973; 1974; 1975) and Glass and Dunlop (1974) reported that several substituted benzoic acids inhibit the absorption of potassium and phosphate by barley roots, thereby affecting the plant growth indirectly. In a study conducted by Jacobson and Jacobson (1980) using excised barley roots, a significant inhibition of respiratory activity and absorption of K^+ and Cl^- were observed when the roots were treated with 2,3,5-triiodobenzoic acid. Salicylic acid (*o*-hydroxybenzoic acid) was also shown to inhibit absorption of K^+ by excised oat roots (Harper and Blake, 1981). Harper and Blake (1981) also reported about 1.6 $\mu mol\ g^{-1}\ hr^{-1}$ uptake of salicylic acid by the excised roots. Depending on the pH of the nutrient solution an uptake of 4-10 mg/g dry weight per hour of ferulic acid and about 1-4 mg/g dry weight per hour of p-hydroxybenzoic acid by cucumber plants was reported by Shann and Blum (1987). A rapid uptake of salicylic acid by sorghum seedlings growing in a nutrient solution was reported by Leather and Einhellig (1988).

To date no systematic study has been conducted on the plant toxicity and uptake of flourobenzoates. However, there are a few reports on the effects of these tracers on some crop plants. Pearson et. al. (1992) reported about 35% growth reduction in barley plants when PFBA (112 kg/ha) and KBr (37 kg Br^-/ha) were applied together in a field test. They also reported reduced barley seed germination in a laboratory test when PFBA and KBr were applied together. Jaynes (1994) reported

a significant decrease in growth of corn and soybean plants when 3,4-DFBA and 3,5-DFBA ($3g/m^2$) were used as tracers. R. C. Rice and coworkers (personal communication, 1992) noticed growth inhibition in immature wheat plants which were exposed to *m*-TFMBA. Nimmo et. al., (1984) reported that 2,6-DFBA, a major degradation product of diflubenzuron, showed no significant uptake by or effect on soybean, cotton or apple plants. All the above studies indicated that a systematic study on plant uptake and plant toxicity of fluorobenzoates needed to be done before these tracers can be used in agronomic situations.

MATERIALS AND METHODS

FLUOROBENZOATES TESTED

Two fluorobenzoate isomers, 2,6-DFBA and 3,4-DFBA, as well as PFBA were used in this study. These were chosen based on their proven usefulness and range of chemical characteristics. PFBA is the most widely used of the fluorobenzoate tracers and has been proven nonreactive in the greatest range of soil and groundwater environments (see Previous Work). Among the difluorobenzoate isomers 2,6-DFBA has been widely used. All the difluorobenzoates have similar properties and appear suitable as groundwater tracers (Bowman & Gibbens, 1992). 3,4-DFBA was also included in this study. 2,6-DFBA (pK_a = 2.85) and 3,4-DFBA (pK_a = 3.83) fall on the extremes (see Table 1) of the pK_a range of all the difluorobenzoate isomers. Except for their pK_as, all the difluorobenzoates have similar physical and chemical properties (Bowman and Gibbens, 1992). It was expected that differences in pK_a would have the greatest effect on differential uptake and toxicity among the isomers. All the three fluorobenzoates were obtained from Yarsley Fluorochemicals Ltd., Wolverhampton U.K., and were used without any further purification.

For the plant uptake study radiolabeled 2,6-DFBA was used along with the non radiolabeled material. The carboxy (14C) labeled 2,6-DFBA (1.68 mci/mmol specific activity and greater then 98% purity) was obtained from Sigma Chemical Company, St Louis, MO, USA.

ANALYTICAL METHOD DEVELOPMENT

The major goal of this work was to study the plant uptake of fluorobenzoate tracers. In order to do this a mass balance for the added tracer needed to be done. This required analyzing for tracer both in soil and plants, the sum of which should be equal to the added amount of tracer per pot (assuming no degradation of tracer). Fluorobenzoates can be analyzed in soil extracts very easily and economically down to μg/L levels via HPLC (Bowman 1984a). But to date noone has reported the analysis of fluorobenzoates in plant tissue. Thus, an analytical method needed to be developed. Any analytical method looking for exotic compounds in plant tissue involves three steps: extraction, sample preparation and quantification. Since there was no previously published method, initial studies were conducted using fluorobenzoate-spiked plant material to validate the extraction and sample preparation. An analytical method was developed to analyze fluorobenzoates in plant material using HPLC.

EXTRACTION OF FLUOROBENZOATES FROM PLANT MATERIAL

Fluorobenzoates used in this study have pK_as ranging from 2.7-3.8 (Table 1) and exist primarily as anions in neutral to basic pH conditions. Due to their low pK_as and high solubilities, vigorous extraction procedures were not deemed necessary. Several published works for the extraction of compounds of similar physical and chemical properties were reviewed. A wide variety of organic acids including phenolic acids (benzoic acids substituted with phenolic group) exist naturally in

plants and play very important roles in plant growth or in protecting the plants or plant parts against fungal attack, or from herbivores. A combination of methanol, acetone, ethanol and/or water are usually used for the extraction of phenolic acids from plant material. Aqueous extraction has been widely used for extraction of anions such as nitrate, chloride, bromide, sulfate and phosphate from plant material (Kalabasi and Tabatabai, 1985; Ouimette and Cofey, 1988). Aqueous extraction was used to extract phenolic acids from plant tissue by Pellissier (1993), and Mole and Joern (1993).

In the present study a hot-water extraction, a methanolic extraction, and a cold-water extraction were tried. Prior to extraction, the plants were rinsed well to remove soil from the roots, were rolled in paper,and dried completely by placing them in a oven at 70° C. The dry weights of the plants were recorded. Plants were ground to a fine powder using a mortar and pestle. This plant powder was used for the extraction of fluorobenzoates.

PREPARATION OF HOT-WATER PLANT EXTRACTS

One gram of finely ground plant material was extracted with 50 mL of Type I water in a Erlenmeyer flask at 60° C on a hot plate, while stirring, for one hour. Type I water for this and all other analyses was prepared using a Mill-Q system (Millipore Corporation, Milford, MA). The extracts were filtered under gravity, using a glass funnel, and the filtrate was used for sample preparation and analysis.

PREPARATION OF METHANOL PLANT EXTRACTS

The methanol extracts were prepared using a method described by Hahn et. al. (1983). Five grams of plant powder was extracted with 20 mL of methanol by keeping the sample on a reciprocating shaker for 30 minutes. The sample was centrifuged and the supernatant was collected. The extraction was repeated 5 times with fresh quantities of methanol. All the extracts were pooled and were reduced to near dryness under vacuum. The residue was brought to 100 mL volume with fresh methanol.

PREPARATION OF COLD WATER PLANT EXTRACTS

Plant powder (0.15 g) was extracted with 50 mL of Type I water by keeping the samples on a reciprocating shaker for about 9 hours at room temperature. Then the plant extracts were filtered under suction with Whatman # 2 filter paper. The filtrate was used for further sample preparation and analysis.

PLANT EXTRACT CLEANUP AND ANALYSIS

Sample preparation is an essential step in the analysis of trace quantities of analytes, especially in complex matrices like plant extracts. Sample preparation is a requirement for several reasons. The most important reasons are to provide the analyte of interest in a solution compatible to further analysis at a concentration that can be detectable without any problems; and to provide a material as clean as possible with minimum interferences especially when using UV detection, in order to prolong

the life of HPLC/GC columns used in the final analysis. In other words sample preparation can be considered as a cleanup and preconcentration step.

Solid-phase extraction, introduced in 1970s, is becoming a widely used method for sample cleanup. Low pressure liquid chromatography is the principle involved behind solid-phase extraction. In solid-phase extraction a small, disposable extraction cartridge filled with sorbent material similar to that of HPLC columns is used. A wide variety of solid-phase extraction columns with different types of sorbent materials are available. Less sample preparation time, a fewer number of steps and therefore less probability of sample loss, and smaller quantities of solvents used are some of the advantages of solid-phase extraction relative to the traditional methods of sample preparation such as liquid-liquid extraction, Soxhlet extraction, and other methods.

Sample cleanup in solid phase extraction can be achieved either by retaining the analyte on the column and selectively eluting the retained analyte using an appropriate solvent, or by retaining the interference matrix on the cartridge and allowing the analyte to pass through.

In this study C18 Sep-Pak ® (Millipore Inc., Milford, MA) solid phase extraction cartridges were used. Table 2 shows the relevant characteristics of the C18 Sep-Paks. The Sep-Paks were preconditioned by passing through them 10 mL of methanol followed by 10 mL of Type I water. Without allowing the cartridge to dry, 10 mL of acidified plant extract was passed through the sep-pak cartridge under suction (approximate flow rate of less than 0.7 mL/min). A vacuum manifold was

Table 2. Features of the Sep-Paks used in this study.
Specifications provided by Millipore, Inc. for c18 Sep-Pak classic.

adsorbent	C18
weight of packing material	360 mg
Hold up volume	0.85 ml
pH	7
% carbon	12
pore size	125 angstroms
particle size	80 μm

used for the sample preparation using solid phase extraction cartridges. The plant extracts were acidified to pH < 1.00 using reagent-grade H_3PO_4. The pH was measured using pH paper. The fluorobenzoates exist in the undissociated state at this low pH and so are retained on the apolar C18 sorbent (Fig. 1). The retained fluorobenzoates were eluted by passing 2-3 mL of 1:1 (v/v) mixture of acetone and phosphate buffer (0.02 M KH_2PO_4 solution, pH adjusted to 2.5 with 0.02M H_3PO_4). The eluent was collected in 20-mL scintillation vials and the volume was measured using a 3 cc syringe. This eluent was used as the sample for chromatography. For samples in which ^{14}C labeled 2,6-DFBA was used, 1 mL of this eluent was used for scintillation counting.

PLANT UPTAKE OF FLUOROBENZOATES

PLANTS STUDIED

Three crop plants were studied in the plant uptake study. They were alfalfa (*Medicago sativa L.*), barley (*Avena sativa L.*) and canola (*Brassica napus L.*). These three plants were selected based on the results of Phase 1 studies. Phase 1 studies were conducted at NMSU, Las Cruces, in order to determine the levels of fluorobenzoates that inhibit the germination of representative crop seeds. The three plants were tested for the uptake of the three previously mentioned fluorobenzoates and bromide. The plants were treated separately with each tracer. Bromide, the well established conservative groundwater tracer, was used as a control.

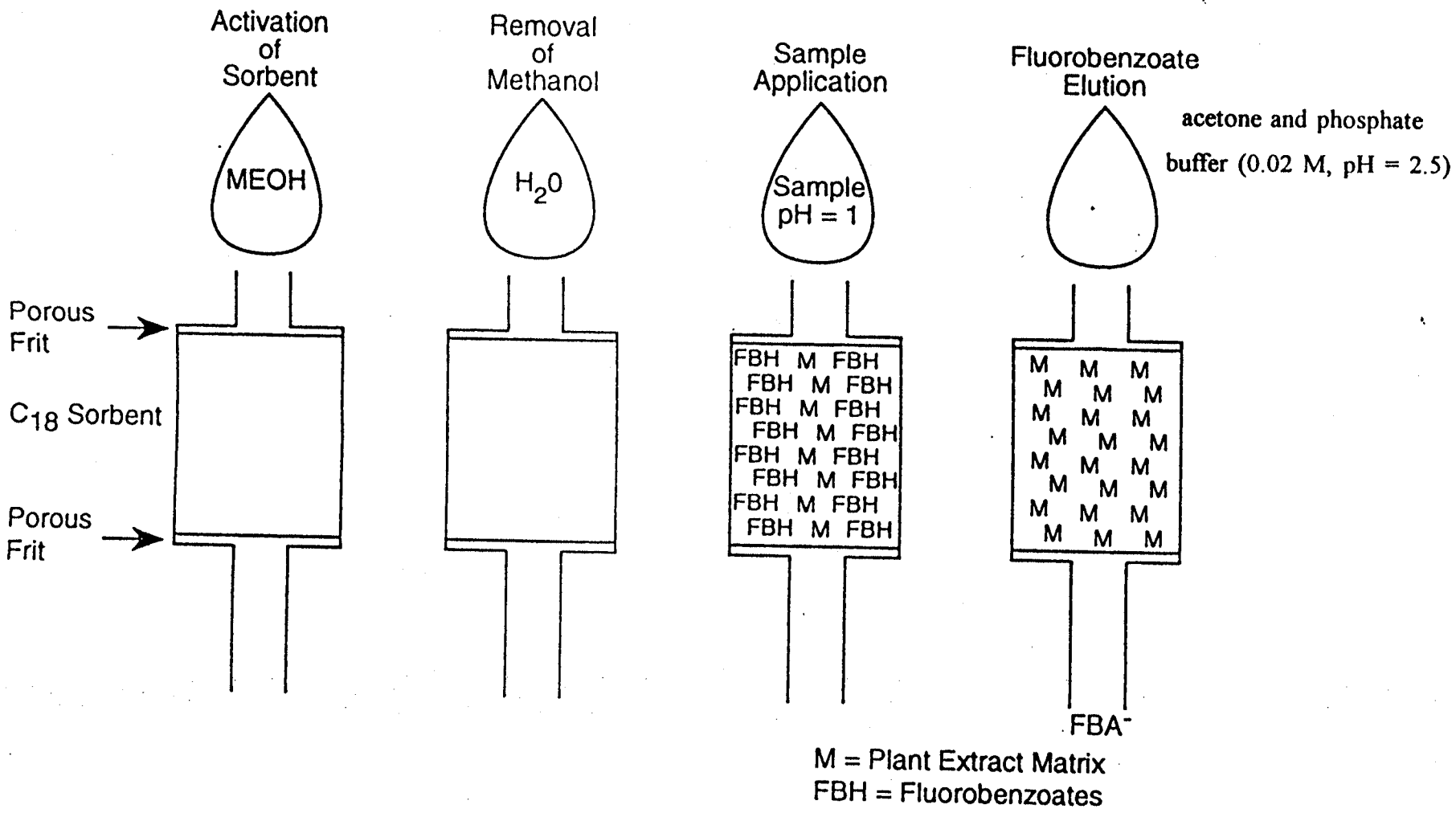


Figure 1. Sample cleanup protocol using acetone and phosphate buffer as eluting solvent.

PLANT GROWTH CONDITIONS

Plants were grown in six-inch diameter pots, with each pot having 600 g of soil. The pots were lined with plastic sheeting to prevent drainage. The soil mixture was prepared by mixing one part Belen soil and three parts sand, both of which were obtained from the Lyndecker research farm, Las Cruces. Based on texture the soil mix in the pots was classified as loamy sand. The composition of the soil was 87% sand, 1% silt and 12% clay. The pH and cation exchange capacity of the soil were 7.2 and 6.6 me/100gms respectively. The organic matter content of the soil was 0.5%.

Alfalfa (Wilson foundation class variety), barley (Schuyler variety) and canola (Cascade variety) were used for this study. Each pot was planted with several seeds of each plant. Each pot was watered with 80 mL of distilled water. The pots were fertilized as needed with a N:P:K::1:2:1 fertilizer. All the pots were watered every day to a constant wet weight. Within the first week of plant growth all the pots were thinned to one plant per pot.

Tracer solution was applied to yield a nominal concentration of 50 mg/L. Twenty five milliliters of a 160 mg/L solution (equivalent to 4 mg) of each tracer solution was added to each pot, resulting in a nominal tracer concentrations in the 80 mL of soil water of 50 mg/L. Each pot/plant was treated with one tracer only. Each treatment was replicated four times. Tracer solutions were applied thirty days after planting for alfalfa, fifteen days after planting for barley, and twenty one days after planting for canola. Plants were allowed to grow further for another two weeks in the case of barley and canola and for one week in the case of alfalfa. Green house

temperatures were maintained at an average of 27°C throughout the growth period of the plants. The plants were grown during the months of May through August 1994.

At this time the plants were harvested by removing the plants carefully from the soil medium and rinsing the roots thoroughly to get rid of any soil material. All the soil along with the rinse water was carefully transferred into plastic ziploc bags, and stored for further analysis. The harvested plants were wrapped in paper and were oven-dried at 70 °C for about one week or until they were completely dry.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS

The dry weight of each plant was recorded. The dried plants were ground to a fine powder using a mortar and pestle. Cold-water extracts of each plant material were prepared using the procedure described in the Materials and Methods section. In the case of plant material treated with ^{14}C labeled 2,6-DFBA, a subsample of the plant powder was analyzed by oxidation and trapping of the CO_2 released. The CO_2 was trapped in 10 mL of scintillation cocktail and was subjected to scintillation counting. All plant material was analyzed for fluorobenzoates as described in the method development section.

The analyses of bromide in plant extracts were done by injecting the plant extracts directly onto the chromatography column (see chromatography section for more details).

PREPARATION OF SOIL EXTRACTS

All the soil along with the rinse water was transferred from the plastic bags into an aluminum baking pan. The plastic bag was rinsed thoroughly three times with Type I water. The rinsed water was added to the baking pan and the soil and water were mixed well using a glass rod. The pan was left in a fume hood until the soil was completely dry. Then the dried soil was carefully scrubbed from the pan and transferred into a ziploc bag. The amount of soil recovered was recorded. The soil was homogenized thoroughly in the bag. Care was taken that there were no lumps present in the soil.

Gravimetric water content of each soil sample was determined as described by Gardener (1986). From each soil sample three 100 g subsamples, were weighed into seperate 500-mL polyethylene centrifuge bottles. One hundred milliliters of Type I water was added to each centrifuge bottle. The bottles were placed on a reciprocating shaker for 24 hours. Then the samples were centrifuged for 30 min at 9000 RPM. The supernatant was carefully decanted into 20 mL scintillation vials. This supernatant was used as the sample for HPLC analysis, and for liquid scintillation counting in the case of radiolabeled 2,6-DFBA samples.

CHROMATOGRAPHY

Both plant and soil extracts were analyzed for the three fluorobenzoates by an anion exchange HPLC method (Bowman, 1984b). The instrumentation consisted of a model 510 HPLC pump, a model U6K manual injector, a model 486 tunable UV-VIS

detector (all from Waters Chromatography Division, Millipore Corporation, Milford, MA) coupled to a Hewlett-Packard ~~5890~~ 3396 integrator/plotter. A 4.6-mm by 250-mm stainless steel analytical column packed with 5-μm Spherisorb strong anion exchange material (Phenomenex, Torrance, CA) was used. Series 800 glass 25-μL syringes from Hamilton Company (Reno, NV) were used for sample injection.

The mobile phase consisted of a 0.02 *M* phosphate buffer mixed with 18 % (v/v) acetonitrile. The phosphate buffer was prepared by using a 0.02 M H_3PO_4 solution to adjust the pH of 0.02 M KH_2PO_4 solution to 2.70. Type I water was used for the preparation of mobile phase. The phosphate buffer was filtered through a 0.45-μm nylon membrane filter, prior to the addition of acetonitrile. A 25-μL sample injection volume was used. The flow rate of the mobile phase was 1.8 mL/min and the detection wavelength was 205 nm.

Analysis of bromide in both soil and plant extracts was accomplished by using a different column and mobile phase (Gerritse and Adeney, 1985). This was due to the coelution of nitrate (used as fertilizer) and bromide peaks while using the above-described column and method. A 4.6-mm by 250-mm stainless steel column packed with a silica bonded quaternary amine (Vydac 302 ion chromatography column, Vydac Separations Group, Hesperia, CA) was used for the analysis of bromide. The mobile phase consisted of 0.02 M KH_2PO_4 buffer, adjusted to a pH of 3.8, using a 0.02M H_3PO_4 solution. The mobile phase flow rate was 1.5 mL/min. The instrumentation and other conditions were the same as those used for the analysis of fluorobenzoates.

RESULTS AND DISCUSSION

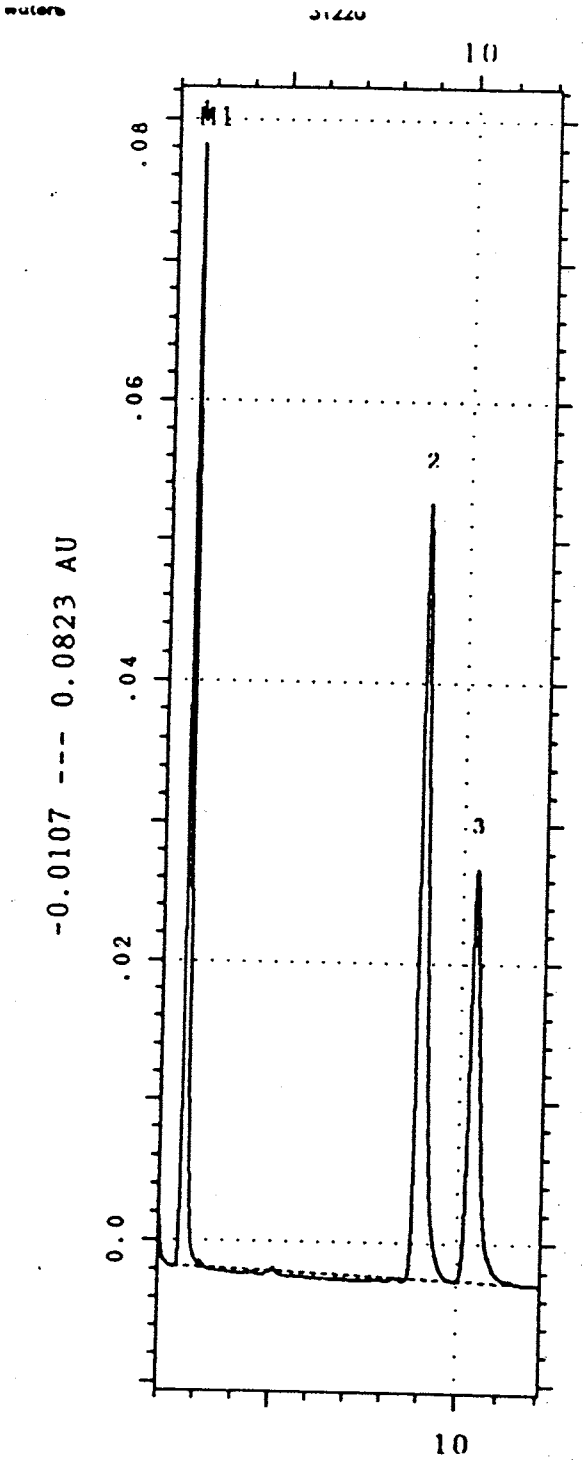
ANALYTICAL METHOD DEVELOPMENT

The three most important steps involved in the method development were (1) extraction of fluorobenzoates from plant tissue, (2) retention of fluorobenzoates on Sep-Pak cartridges, and (3) elution of retained fluorobenzoates from the cartridge.

COMPARISION OF EXTRACTION METHODS

There was no previous work done regarding the extraction of fluorobenzoates from plant material. However, several workers were able to extract phenolic acids from plant material using aqueous extraction (see Materials and Methods). Initial studies were conducted to determine the feasibility of extraction using hot water and methanol. A good extraction technique should be able to extract the compounds of interest from plant material efficiently, with a minimum amount of interference so that the sample preparation steps will be fewer. The hot water and methanol extracts were studied to know how the chromatograms of these extracts look relative to a chromatogram of a standard solution.

Figure 2 shows a chromatogram of a standard solution (20 mg/L in water) of the three fluorobenzoates used in this study. If any extract is reasonably clean with little interference and does not have any effect on the sensitivity of detection of analytes at trace quantities, that extraction technique can be used. Figures 3,4, and 5 show the chromatograms of hot water extracts of cotton, chilli and alfalfa without added fluorobenzoates. The resultant chromatograms are very complex with a number



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	2.70	0.08000	2.42	3.09	9.614E-03	31.6	
2	8.98	0.05525	3.09	9.82	1.217E-02	40.0	V
3	10.35	0.02954	9.94	12.04	8.642E-03	28.4	I

No.	Sample name	Window	Base	Amount	Purity	Match	Library
1	34DFBA	0.00	HEIGHT	2.025E+01	****	0/ 990	******
2	26-DFBA	0.01	HEIGHT	2.019E+01	****	0/ 799	4/ 948
3	PFBA	0.03	HEIGHT	2.025E+01	****	0/ 774	7/ 911

Figure 2. Chromatogram of a three tracer standard in water.

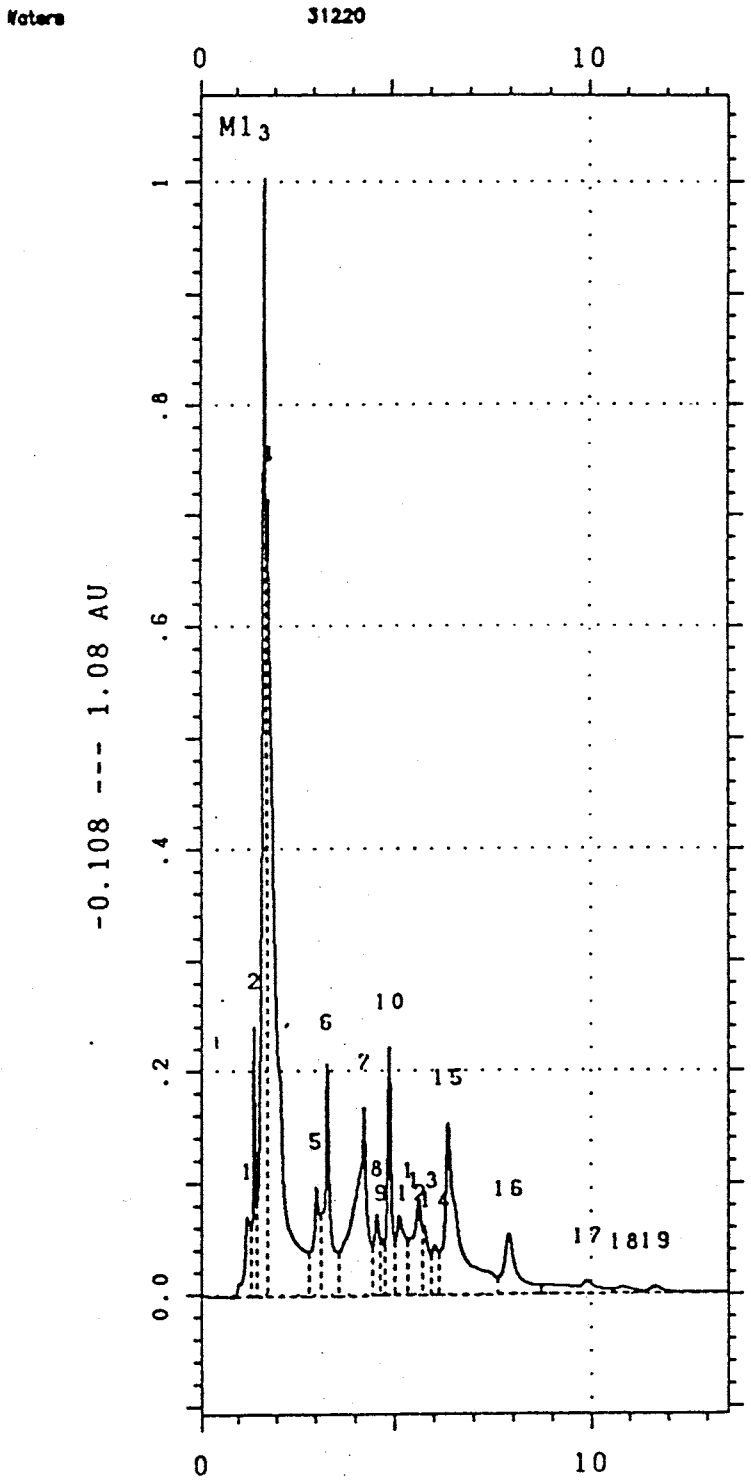


Figure 3. Chromatogram of hot-water plant extract of cotton.

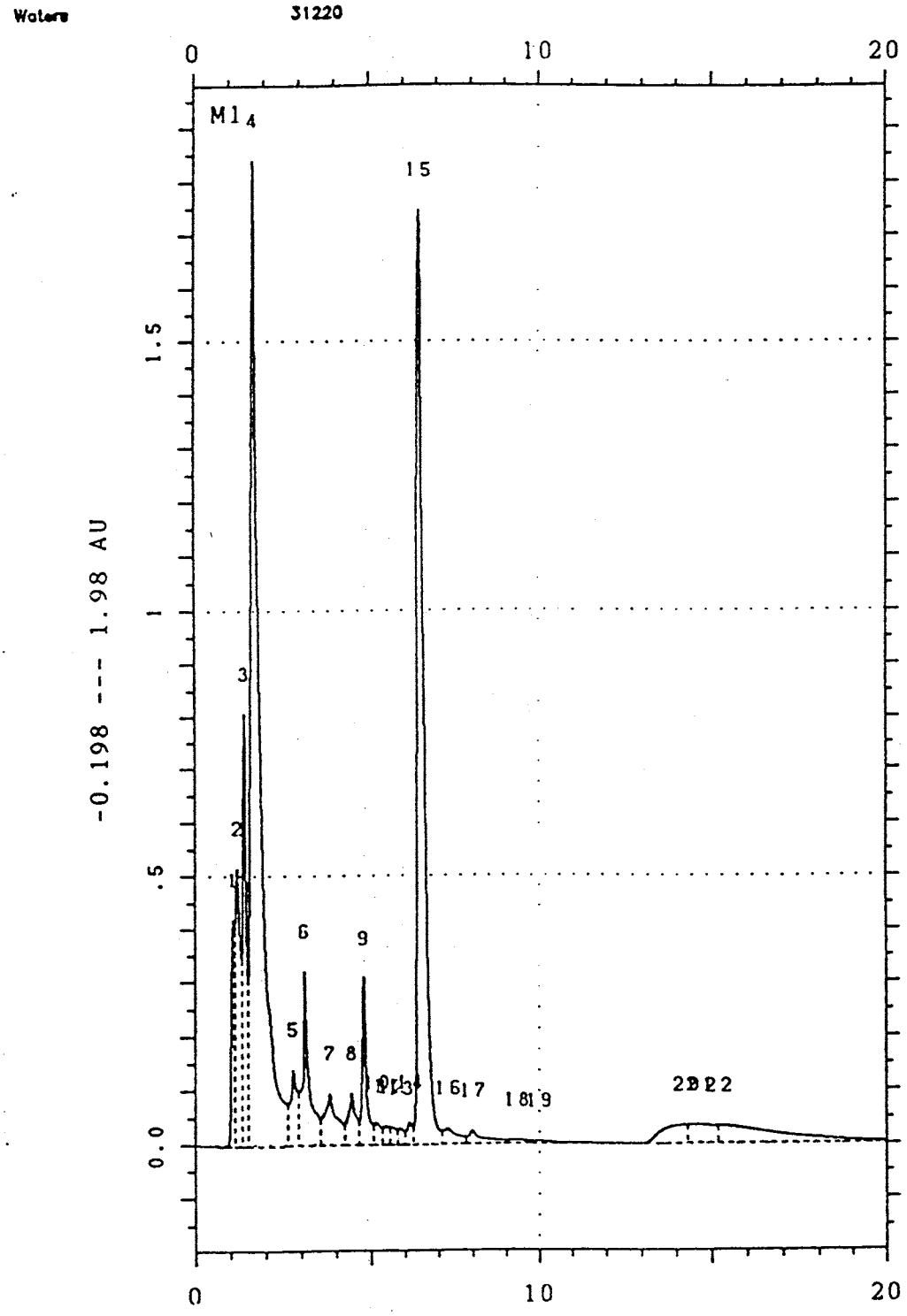


Figure 4. Chromatogram of hot-water plant extract of chile.

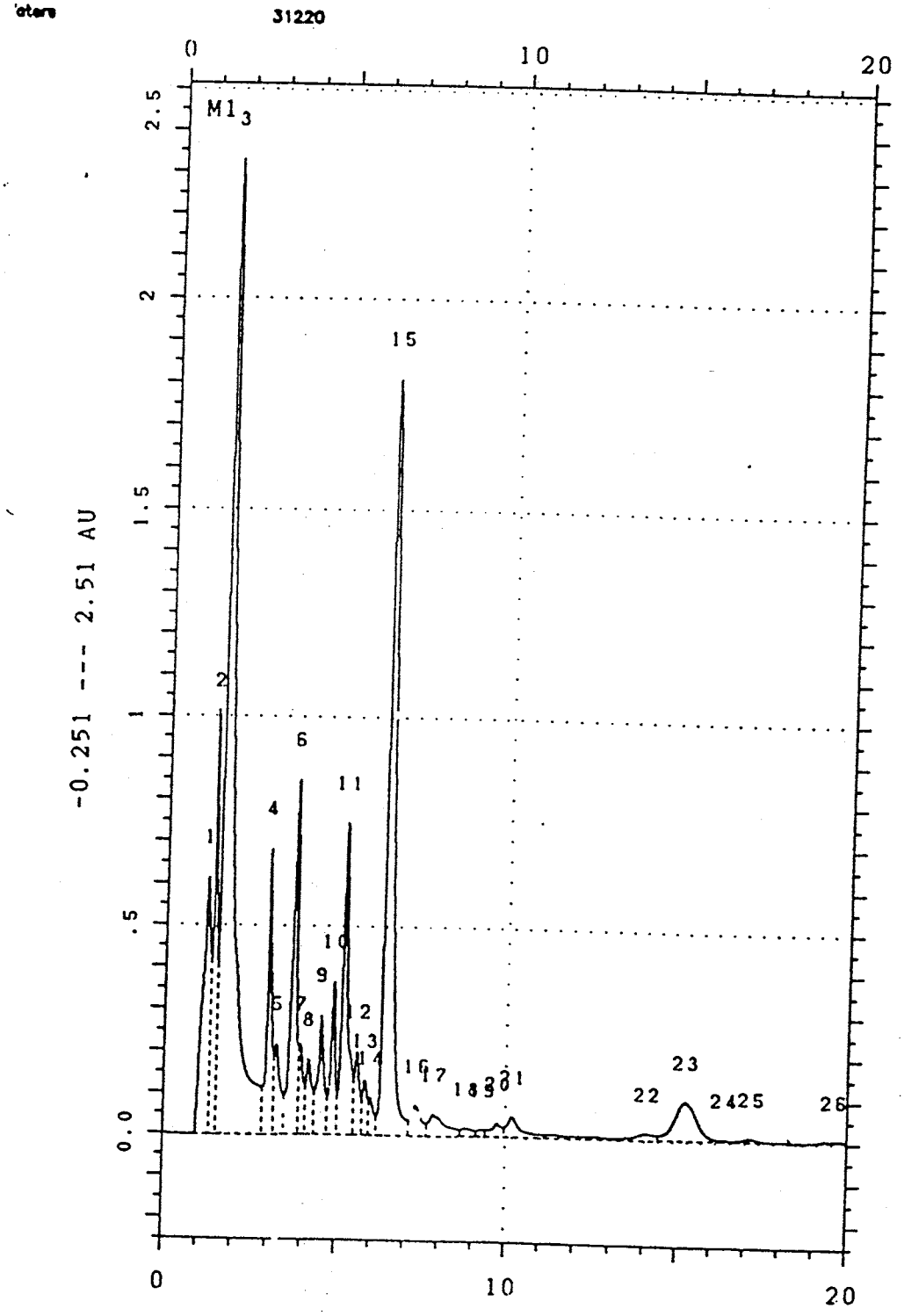


Figure 5. Chromatogram of hot-water plant extract of alfalfa.

of peaks and high absorbance at the wavelength of detection. The methanol extracts of these same plants looked very dark green in color. Methanol, being a good organic solvent, extracts many organic compounds from plant material. This makes the sample preparation steps complex and may result in error. Due to the quantities of organic soultes they generated, the methanol and hot water extracts were not studied further.

VALIDATION OF COLD WATER EXTRACTION METHOD

The cold water extraction technique was validated by quantifying ^{14}C-labeled 2,6-DFBA in the plant tissue from the plant uptake study. Since all fluorobenzoates have similar physical and chemical properties, an extraction technique that works well with 2,6-DFBA was expected to extract other fluorobenzoates also.

Plants from the treatments which included ^{14}C-labeled 2,6-DFBA were analyzed by two techniques : (1) oxidation of ground plant material and collection of the $^{14}CO_2$, and (2) aqueous extraction. If the aqueous extraction was efficient, the results obtained from ^{14}C counting in both studies should result in complete recovery of added 2,6-DFBA. Tables 3, 4 and 5 show the comparisons between the results obtained by oxidation technique and aqueous extraction, for alfalfa, barley and canola plant samples along with the results of comparisions of the means using a t-test. Percent recoveries were calculated relative to the total activity applied. As can be seen from Tables 3,4 and 5, the t-values in all the cases were below the critical t-values. This shows that the null hypothesis of t-test (means obtained from the two methods are

Table 3. Comparision of recoveries of 14C-labelled 2,6-DFBA, by aqueous extraction and oxidation of alfalfa plant samples. The means of the two methods were not significantly different at the P = 0.05 level (t = 0.174124)

		AQUEOUS EXTRACTION				OXIDATION			
Sample name	Total plant mass (g)	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery
A106	0.30	886667	266000	2925470	9	1166673	350001	3258100	10.7
A204	0.30	1287667	386300	2925470	13	1542833	462850	3258100	14.2
A306	0.24	1005000	241200	2925470	8	735460	176510	3258100	5.4
A404	0.28	1626333	455373	2925470	15.5	1198111	335471	3258100	10.3
	Average :	1201417			11.375	1160769			10.15

Table 4. Comparision of recoveries of 14C-labelled 2,6-DFBA, by aqueous extraction and oxidation of barley plant samples
The means of the two methods were not significantly different at the P = 0.05 level (t = -2.05521)

		AQUEOUS EXTRACTION				OXIDATION			
Sample name	Total plant mass (g)	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery
B106	0.492	26628034	13100993	60862125	22	37431990	18416539	63053918	29
B204	0.416	36181167	15051366	60862125	25	45831293	19065817	63053918	30
B304	0.424	29999216	12719668	60862125	21	37731871	15998313	63053918	25
B402	0.218	40975280	8932611	60862125	15	52736735	11496608	63053918	18
	Average :	33445924			20.75	43432973			26

Table 5. Comparision of recoveries of 14C-labelled 2,6-DFBA, by aqueous extraction and oxidation of canola plant samples
The means of the two methods were not significantly different at the P = 0.05 level (t = -1.26107)

		AQUEOUS EXTRACTION				OXIDATION			
Sample name	Total plant mass (g)	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery	CPM / g of plant matter	Total CPM recovered	CPM applied	% recovery
		AQUEOUS EXTRACTION				OXIDATION			
C106	2.59	11278683	29211790	60862125	48	15237473	39465056	65236181	61
C206	2.58	10259533	26469596	60862125	43	9941506	25649087	65236181	39
C304	2.60	10477250	27240850	60862125	45	12951023	34449722	65236181	53
C407	3.00	8854200	26562600	60862125	44	9800613	29401840	65236181	45
	Average :	10217417			45	11982654			50

same) is not invalidated and that the two methods give comparable results. The recoveries are quite comparable and suggest that cold-water extraction is a good technique for extraction of fluorobenzoates from the plant material.

The efficiency of aqueous extraction was checked using the ratio of %recovery (oxidation technique) to % recovery (aqueous extraction). The values obtained were (as percentages) 112 %, 80% and 90% for alfalfa, barley and canola respectively. In a similar way extraction efficiency was also checked by using the ratios of DPM/g of plant material values. These values were 104%, 77% and 85% for alfalfa, barley and canola plants respectively. These results indicate that aqueous extraction is a good technique for extraction of fluorobenzoates from plant material.

VALIDATION OF EXTRACT CLEANUP METHOD

Retention of fluorobenzoic acids on Sep-Paks is controlled by the sample pH. Fluorobenzoates (PFBA, *m*-TFMBA) were successfully retained on a reversed-phase packing material similar to Sep-Paks, for trace enrichment by Stetzenbach et. al. (1982). Organic acids can be retained on reverse phase adsorbent media if they exist in the protonated state, due to their higher affinity for the similar medium and their poor solubility in water. However, for the organic acids to exist in protonated state the pH of the sample should be at least 2 units below the pK_a of the organic acids (Stetzenbach et. al., 1982). For this reason the sample pH was brought down to 1 by adding reagent grade H_3PO_4 before passing it through the Sep-Pak cartridge. A similar technique was used by Moors et. al., (1991) for the cleanup of various food

samples, and the quantitative determination of benzoic acid used as a preservative. In the case of plant extract samples involving ^{14}C-labeled 2,6-DFBA, the waste coming out from the Sep-Pak cartridge during the fluorobenzoate retention step was checked for ^{14}C activity. Table 6 shows these values for the three plant samples used in this study. The %DPM values in the waste coming out from the sep-pak were negligible. This indicates that 2,6-DFBA was retained queit well on the Sep-Paks.

To elute the retained fluorobenzoates from the Sep-Paks an eluent with the appropriate combination of organic solvent, ionic strength and pH should be used. The eluent used should be strong enough to be able to elute the fluorobenzoates from the cartridge with a minimum volume of solvent, it should be compatible with the mobile phase so that it can be injected directly into the HPLC, and it should elute a minimum amount of interfering matrix. Moors et. al., (1991) used a methanol and NH_4OH (0.02M) combination to elute benzoic acid retained on C18 packing material.

Initial studies were conducted using spiked plant extracts and standard solutions using the methanol-NH_4OH solvent. Figure 6 shows the sample elution protocol used for the initial studies. These studies were conducted using wheat, cotton, and alfalfa plant extracts. The sample volume and the eluting solvent volume (5ml) were same. This was done to make the estimation of recoveries easier and to avoid any possibility of concentrating the interfering material. Figures 7, 8, 9 and 10 show the chromatograms along with the corresponding recoveries. Even though this eluent gave good recoveries, methanol being a very good solvent, there is a possibility that it may bring out lot of interference material if this solvent is used for

Table 6. CPM values of waste coming out from Sep-Pak showing the retention effeciency of 2,6-DFBA on Sep-Paks.

Sample name	CPM/10ml of sample Before clean up	CPM/10 ml of waste collected eluted from sep-pak	% CPM in waste
A106	26600	151.50	0.6
A204	38630	100.15	0.3
A306	30150	105.1	0.4
A404	48790	86.45	0.2
Mean	36042	110.8	0.38
Std. Devn	9884	28.3	0.17
B106	798841	2970.4	0.4
B204	1085435	2759.7	0.3
B304	899976	2803.6	0.3
B402	1249495	2322.85	0.2
Mean	1008436	2714	0.3
Std. Devn	199776	276	0.08
C106	338360	1100.25	0.3
C206	307786	932.05	0.3
C304	314317	946.70	0.3
C407	259865	943.60	0.4
Mean	305082	981	0.33
Std. Devn	32887	80	0.05

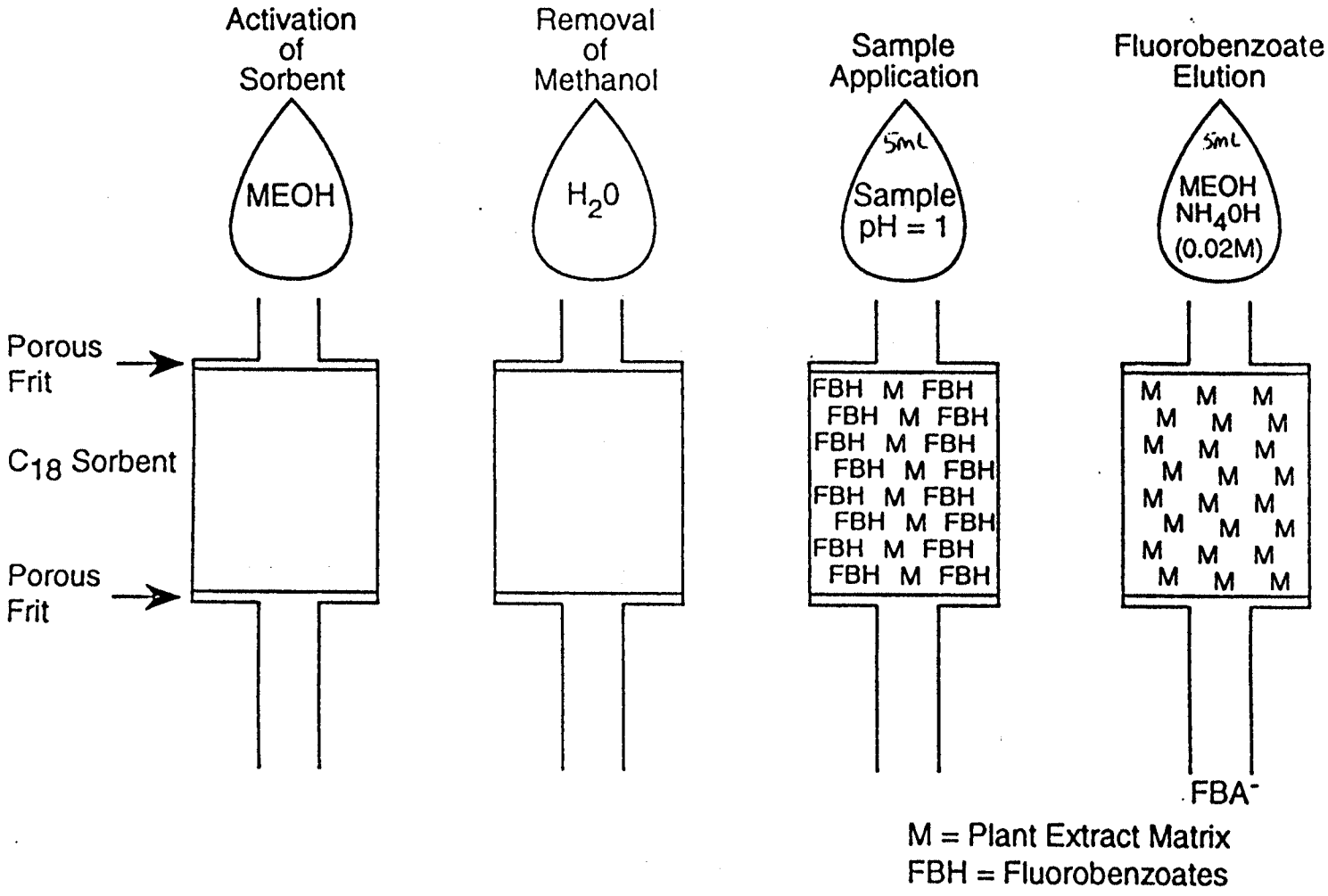
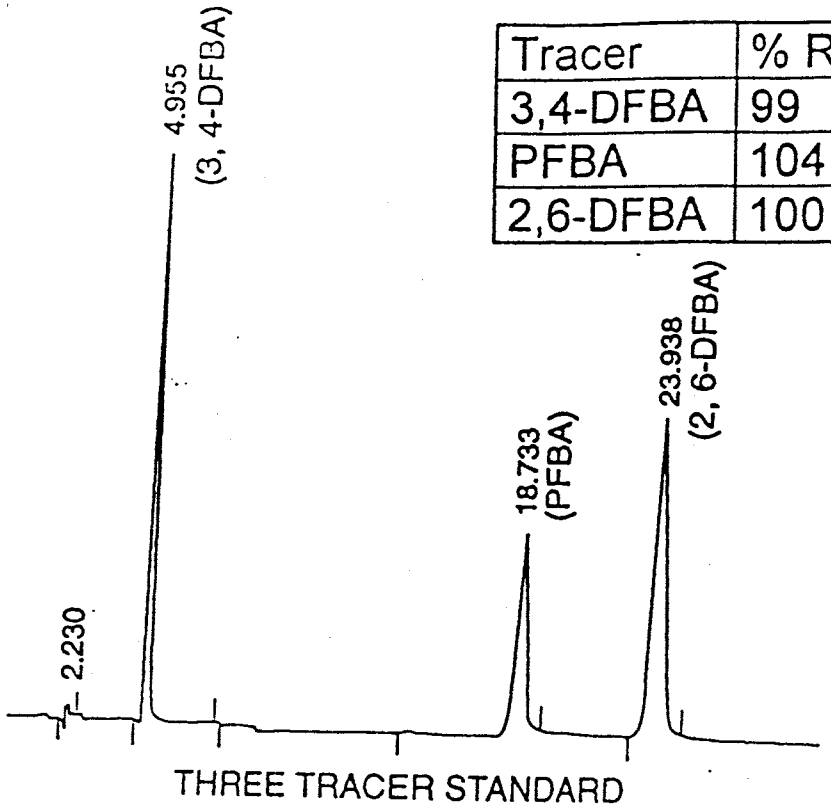


Figure 6. Sample cleanup protocol using Methanol and NH_4OH as eluting solvent.

Tracer	% Recovery
3,4-DFBA	99
PFBA	104
2,6-DFBA	100



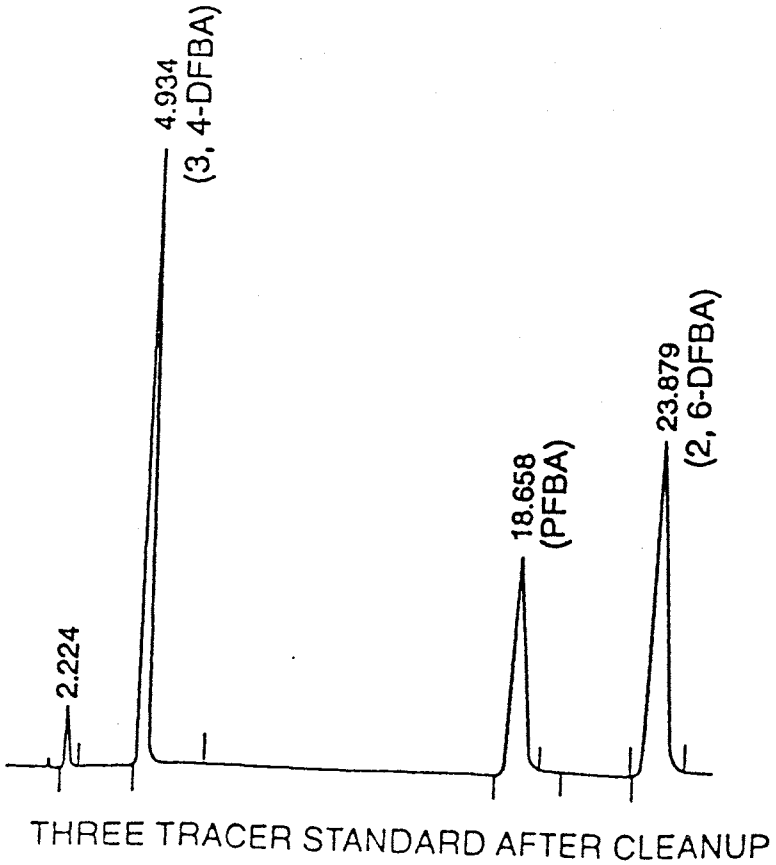
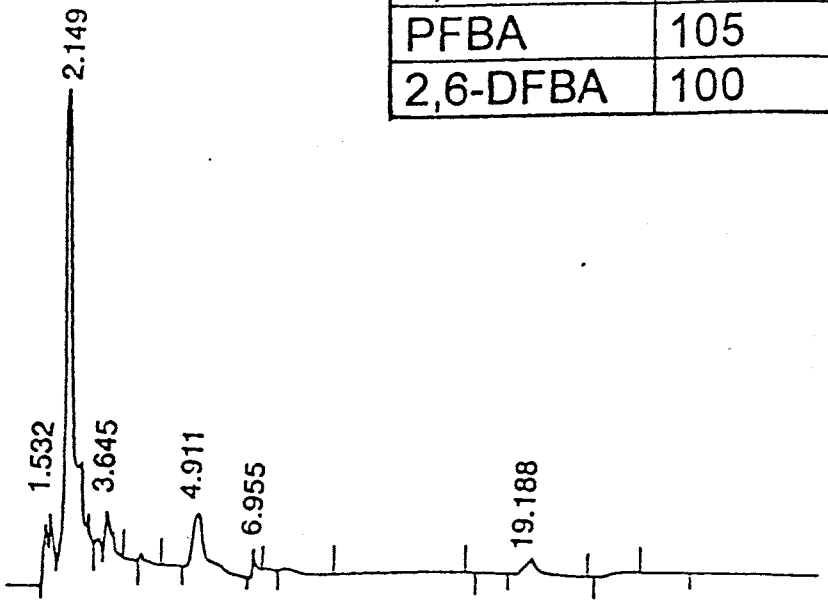
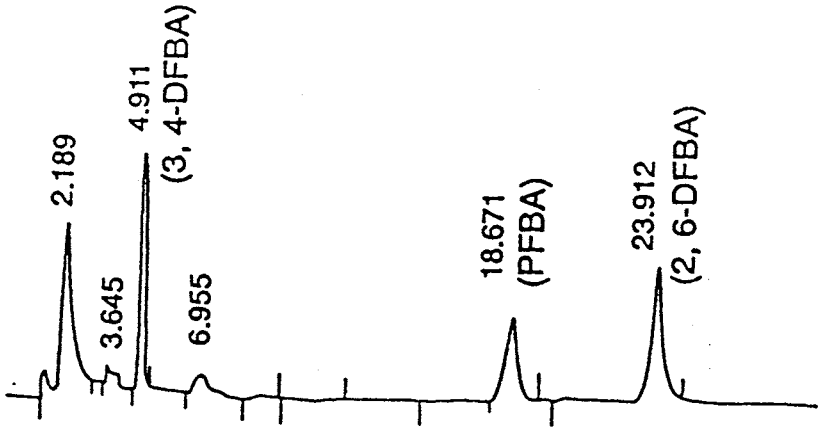


Figure 7. Tracer recovery after sample cleanup of a standard solution using Methanol/NH_4OH as eluting solvent.

Tracer	% Recovery
3,4-DFBA	115
PFBA	105
2,6-DFBA	100



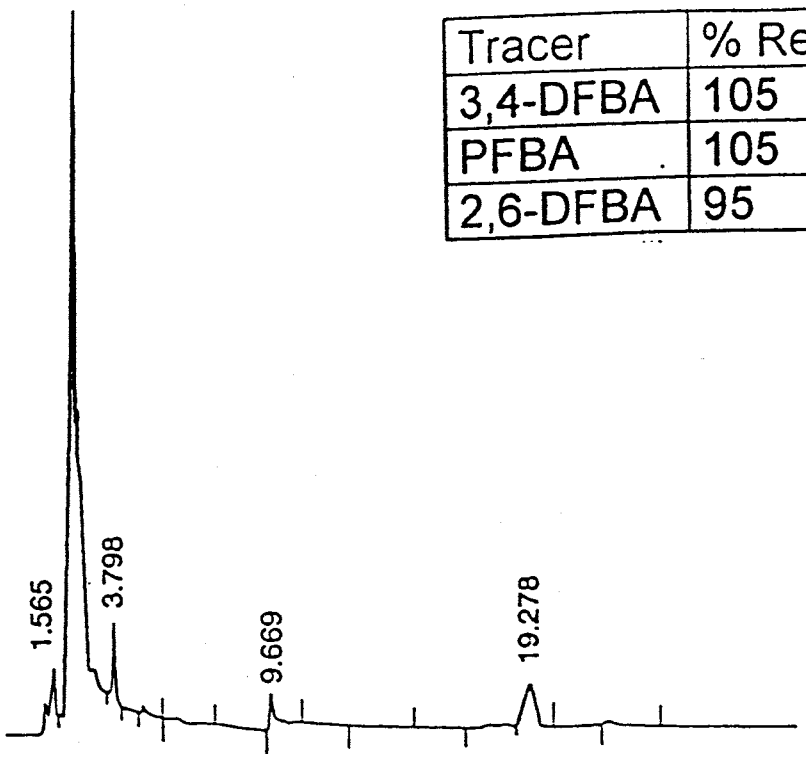
RAW WHEAT PLANT EXTRACT



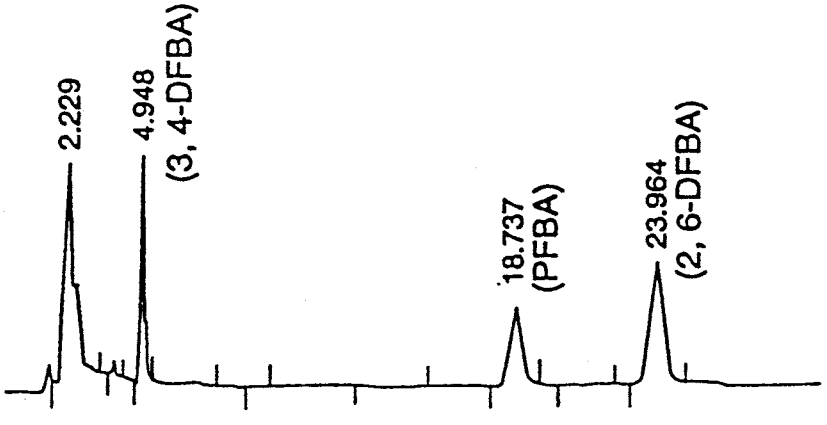
WHEAT EXTRACT SPIKED WITH 20 mg./L of TRACERS
(AFTER CLEANUP)

Figure 8. Tracer recovery from a spiked wheat plant extract after sample cleanup using Methanol/NH_4OH as eluting solvent.

Tracer	% Recovery
3,4-DFBA	105
PFBA	105
2,6-DFBA	95



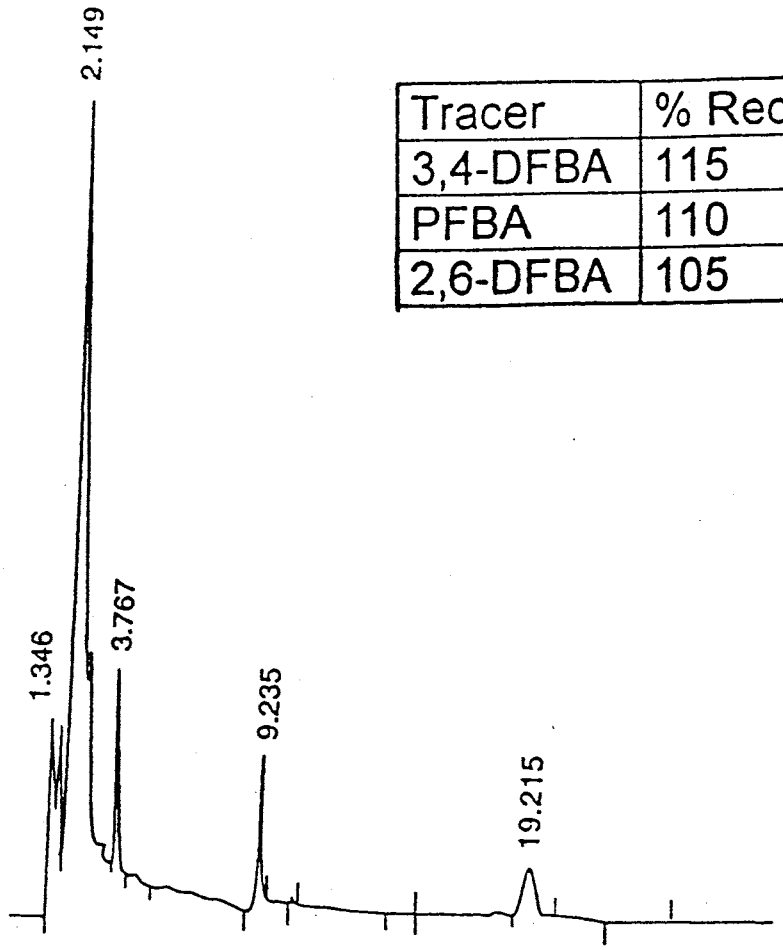
COTTON RAW PLANT EXTRACT



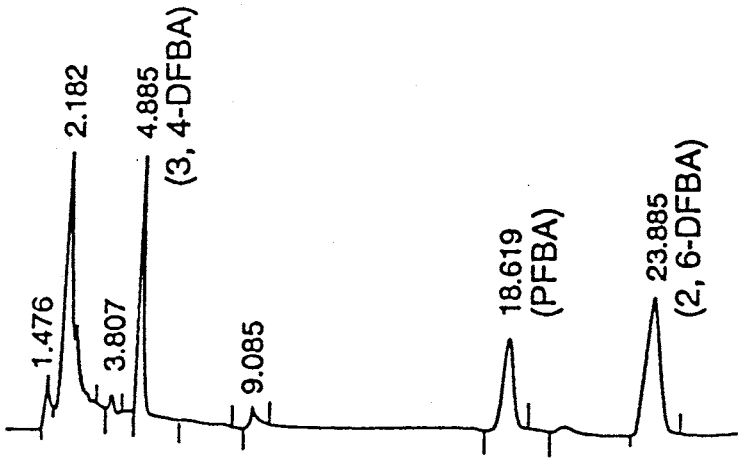
COTTON EXTRACT SPIKED WITH 20 mg/L OF TRACERS
(AFTER CLEANUP)

Figure 9. Tracer recovery from a spiked cotton plant extract after sample cleanup using Methanol/NH_4OH as eluting solvent.

Tracer	% Recovery
3,4-DFBA	115
PFBA	110
2,6-DFBA	105



ALFALFA RAW PLANT EXTRACT



ALFALFA EXTRACT SPIKED WITH 20 mg./L OF TRACERS
(AFTER CLEANUP)

Figure 10. Tracer recovery from a spiked alfalfa plant extract after sample cleanup using Methanol/NH_4OH as eluting solvent.

concentration of fluorobenzoates. This was noticed when an actual sample of canola plant treated with 2,6-DFBA was analyzed by this way. The sample showed good recovery in terms of DPM values but when injected into HPLC the peak of interest was not resolved due to large amount of interference.

For the actual plant samples 2-3 ml of a 1:1 mixture of acetone and phosphate buffer (0.02 M, pH = 2.5) was used as the eluent. Acetone is a good solvent for double bonded compounds and was expected to be able to elute the fluorobenzoates from the Sep-Paks. The low pH of the buffer prevented the pH of the cartridge from increasing, so the major organic interferents would be retained in the cartridge.

Table 7 shows the percent recoveries obtained when standard solutions and spiked alfalfa, barley and canola plant extracts were subjected to the above mentioned sample cleanup method. The plant material containing no tracers was spiked with a known quantity of tracer either PFBA or 2,6-DFBA and was ground together. The low retention times of 3,4-DFBA resulted in the non resolution of that peak from the solvent peak. For this reason no data is available for 3,4-DFBA in plant extracts. This spiked plant material/powder was subjected to the sample cleanup protocol as described in the Materials and Methods section. The recoveries ranged from 84 to 98%. Besides the pH of sample, the other factor controlling the retention and elution was flow rate. All the sample preparation was done using a vacuum manifold, and care was taken that a very low flow rate (less then 1ml per minute) was maintained for the better retention of the fluorobenzoates.

The sample cleanup protocol was also validated by using the CPM values from the plant samples treated with ^{14}C-labeled 2,6-DFBA. Percent recoveries were

Table 7. Recoveries from standards and spiked plant extracts subjected to sample cleanup
(sample name reflects tracer spiked and S = standard, A = alfalfa,
B = barley, C = canola, n = number of repetitions)

Sample name	Amount spiked (μg/10ml extract)	Average amount recovered μgms (n)	Standard. deviation (c.v %)	% recovery
SPFBA	20	17.45 (5)	0.60 (3.4)	87
APFBA	20	16.80 (3)	1.56 (9.3)	84
BPFBA	20	18.25 (3)	1.63 (8.9)	91
CPFBA	20	18.55 (3)	1.12 (6.0)	93
S2,6-DFBA	10	8.83 (4)	0.73 (8.3)	88
A2,6-DFBA	10	9.13 (3)	0.51 (5.6)	91
B2,6-DFBA	10	9.60 (3)	0.36 (3.8)	96
C2,6-DFBA	10	9.76 (3)	0.57 (5.8)	98

calculated in terms of CPM values relative to the CPM values of the plant extracts/samples prior to the sample clean up protocol (total CPM applied to the Sep-Pak versus total CPM recovered from Sep-Paks). Table 8 shows these percent recoveries obtained from liquid scintillation counting of these samples. The recoveries ranged from 85-95%.

PLANT UPTAKE AND PLANT TOXICITY

Tables 9, 10 and 11 show the recoveries of the tracers from the analysis of soil extracts of the alfalfa, barley and canola studies. Highest average recoveries were obtained for bromide. This was followed by PFBA and 2,6-DFBA. However, in the case of alfalfa 2,6-DFBA recovery was more than that of PFBA. The average recoveries of bromide were 91%, 109%, and 48% for alfalfa, barley and canola soil samples respectively. For PFBA the average recoveries were 72%, 69%, and 51% for alfalfa, barley and canola soils respectively. The average recoveries for 2,6-DFBA were 83%, 59%, and 30%, for the alfalfa, barley and canola soils respectively. The average recoveries for 3,4-DFBA were 39%, 42%, and 34%, for the alfalfa, barley and canola soils respectively.

Amongst the various fluorobenzoates PFBA is considered to be the most stable due to the greater number of fluorine substitutions on the ring. This high stability (chemical stability due to five substituted highly electronegative fluorine atoms) and its size may be the reasons for its lower uptake by plants.

Assuming that missing mass was taken up by plants, 2,6-DFBA shows higher uptake by the plants relative to PFBA. The recoveries from the analysis of plant

Table 8. CPM values showing recoveries of 14C-labelled 2,6-DFBA samples of Alfalfa (A), barley (B) and canola (C) subjected to sample cleanup

Sample name	CPM/ml after cleanup		Average	% recovery
	Trial 1	Trail 2		
A106	12032	11989	12010	90
A204	18800	18757	18779	97
A306	14422	14389	14405	96
A404	23182	23265	23223	95
Mean	17109	17100	17104	94.5
Std. Devn	4924	4974	4949	3.1
B106	337730	360414	349072	87
B204	463844	487079	475462	88
B304	387089	408940	398015	88
B402	538543	576881	557712	89
Mean	431801	458328	445065	88
Std. Devn	88070	94706	91363	0.82
C106	154294	167631	160963	95
C206	130128	130836	130482	85
C304	134050	137294	135672	86
C407	113891	118464	116178	89
Mean	133090	138556	135824	88.75
Std. Devn	16612	20898	18677	4.5

Table 9. Results of the analysis of soil extracts of alfalfa samples

Sample name	Treatment	Amount of soil recovered (g)	Amount of tracer recovered (mg/tot. soil)			Average (mg)	% recovered
			Trial 1	Trial 2	Trial 3		
A103	BROMIDE	599.88	2.95	2.56	2.45	2.65	92.7
A203	BROMIDE	598.93	2.30	2.30	2.65	2.42	84.6
A302	BROMIDE	599.11	2.55	2.43	3.20	2.73	95.5
A405	BROMIDE	599.46	2.68	2.37	2.60	2.55	89.2
Mean		599.35	2.62	2.42	2.73	2.59	90.5
Std. Dev		0.42	0.27	0.11	0.33	0.13	4.67
A105	PFBA	599.89	2.67	2.94	2.81	2.81	70.3
A202	PFBA	599.16	3.38	1.87	2.35	2.53	63.3
A305	PFBA	599.50	2.41	2.97	2.83	2.74	68.5
A403	PFBA	599.80	2.95	4.16	3.12	3.41	85.3
Mean		599.59	2.85	2.99	2.78	2.87	71.8
Std. Dev		0.33	0.42	0.94	0.32	0.38	9.42
A106	2,6-DFBA	599.60	4.05	4.32	4.33	4.23	91.8
A204	2,6-DFBA	599.61	3.46	3.38	3.46	3.43	74.4
A306	2,6-DFBA	600.40	4.15	4.05	3.98	4.06	88.1
A404	2,6-DFBA	596.62	3.80	3.40	3.60	3.60	78.1
Mean		599.10	3.87	3.79	3.84	3.83	83.10
Std. Dev		1.67	0.31	0.47	0.39	0.38	8.17
A104	3,4-DFBA	599.71	1.39	1.50	1.48	1.46	36.5
A201	3,4-DFBA	598.50	1.47	1.47	1.45	1.46	36.5
A307	3,4-DFBA	599.30	1.66	1.59	1.80	1.68	42.0
A406	3,4-DFBA	599.32	1.65	1.47	1.62	1.58	39.5
Mean		599.21	1.54	1.51	1.59	1.55	38.63
Std. Dev		0.51	0.13	0.06	0.16	0.11	2.66

Table 10. Results of the analysis of soil extracts of barley samples.

Sample name	Treatment	Amount of soil recovered (g)	Amount of tracer recovered (mg/tot. soil)			Average (mg)	% recovered
			Trial 1	Trial 2	Trial 3		
B103	BROMIDE	598.38	2.80	2.92	3.26	2.99	107.4
B201	BROMIDE	596.80	2.64	2.85	2.70	2.73	98
B303	BROMIDE	601.59	2.63	2.76	3.03	2.81	100.9
B406	BROMIDE	598.57	3.33	3.81	3.76	3.63	130.3
Mean		598.84	2.85	3.09	3.19	3.04	109.2
Std. Dev		2.00	0.33	0.49	0.45	0.41	14.64
B105	PFBA	596.70	2.22	2.69	2.18	2.36	59
B207	PFBA	597.90	3.12	2.57	2.69	2.79	69.8
B306	PFBA	598.90	2.41	2.96	2.67	2.68	67
B401	PFBA	599.10	2.81	3.39	3.20	3.13	78.3
Mean		598.15	2.64	2.90	2.69	2.74	68.5
Std. Dev		1.1	0.40	0.36	0.42	0.32	7.94
B106	2,6-DFBA	601.33	2.59	2.15	2.01	2.25	56.3
B204	2,6-DFBA	601.06	1.82	2.31	2.45	2.19	54.8
B304	2,6-DFBA	601.17	2.45	2.42	2.10	2.32	58
B402	2,6-DFBA	601.31	2.53	2.64	2.70	2.62	65.5
Mean		601.22	2.35	2.38	2.32	2.35	58.63
Std. Dev		0.13	0.36	0.21	0.32	0.19	4.77
B104	3,4-DFBA	599.40	1.73	1.23	1.71	1.56	39
B205	3,4-DFBA	599.50	1.47	1.44	1.32	1.41	35.3
B301	3,4-DFBA	599.23	1.92	1.86	1.87	1.88	47
B403	3,4-DFBA	597.11	2.03	1.82	1.69	1.85	46.3
Mean		598.81	1.79	1.59	1.65	1.68	41.88
Std. Dev		1.14	0.25	0.30	0.23	0.23	5.7

Table 11. Results of the analysis of soil extracts of canola samples.

Sample name	Treatment	Amount of soil recovered (g)	Amount of tracer recovered (mg/tot. soil)			Average (mg)	% recovered
			Trail 1	Trail 2	Trail 3		
C103	BROMIDE	617.94	1.40	1.58	1.41	1.46	52
C203	BROMIDE	597.31	1.27	1.35	1.25	1.29	46
C302	BROMIDE	599.14	1.55	1.27	1.20	1.34	48
C404	BROMIDE	598.59	1.16	1.29	1.36	1.27	46
Mean		603.25	1.35	1.37	1.31	1.34	48
Std. Dev		9.83	0.17	0.14	0.10	0.09	2.83
C105	PFBA	599.32	1.55	1.61	1.62	1.59	39.75
C202	PFBA	598.76	1.64	1.76	2.17	1.86	46.50
C303	PFBA	598.97	2.49	1.88	2.42	2.26	56.50
C403	PFBA	598.04	2.24	2.24	2.75	2.41	60.25
Mean		598.77	1.98	1.87	2.24	2.03	50.75
Std. Dev		0.54	0.46	0.27	0.48	0.37	9.35
C106	2,6-DFBA	598.76	1.31	1.43	1.28	1.34	33.50
C206	2,6-DFBA	595.97	1.37	1.28	1.42	1.36	34
C304	2,6-DFBA	590.61	0.83	0.82	0.83	0.83	20.75
C407	2,6-DFBA	599.39	1.41	1.29	1.22	1.31	32.75
Mean		596.18	1.23	1.21	1.19	1.21	30.25
Std. Dev		4.00	0.27	0.27	0.25	0.25	6.35
C104	3,4-DFBA	599.23	1.55	1.59	1.49	1.54	38.50
C201	3,4-DFBA	597.62	1.00	1.36	1.21	1.19	29.75
C305	3,4-DFBA	598.36	1.57	1.44		1.51	37.75
C406	3,4-DFBA	597.07	1.41	0.99	1.02	1.14	28.5
Mean		598.07	1.38	1.35	1.24	1.35	33.63
Std. Dev		0.94	0.26	0.26	0.24	0.21	5.23

extracts also reflect this. Tables 12,13 and 14 show the results of the analysis of alfalfa, barley and canola plant extracts, respectively, for bromide, 2,6-DFBA, and PFBA. 3,4-DFBA was not determined due to its coelution with the solvent peak or some inerfernce material. The short retention time of 3,4-DFBA (see fig. 2) may be the reason for this problem.

Maximum recoveries from plant extracts were obtained for 2,6-DFBA of alfalfa and barley plants, and for bromide in the case of canola plants. Average percent recoveries values for bromide were 2.3%, 5.1%, and 55% for alfalfa, barley and canola plant samples respectively. 2,6-DFBA showed an average recovery of 8.6%, 22%, and 49% for alfalfa, barley and canola samples respectively. An average recovery of 0.10%, 1.7%, and 8.7% were obtained for PFBA from the alfalfa, barley, and canola plant samples respectively.

There is a huge variability in the amount of uptake of the three compounds amongst the three species of plants. Canola showed maximum uptake of the three compounds, followed by barley and alfalfa. A significant and direct relation can be noticed between the degree of uptake and the plant mass. Alfalfa plants had the minimum plant mass (dry weight) and correspondingly showed minimum uptake of the three compounds.

The partitioning of the fluorinated benzoic acids between the water and plant material may be explained on the basis of their octanol-water partition coefficients (log K_{ow}) and their pK_as. Log K_{ow} values were estimated for the 2,6-DFBA and PFBA using Leo's fragment constant method (Lyman et. al., 1982). The estimated log K_{ow}

Table 12. Results of the analysis of alfalfa plant extracts.

Sample name	Treatment	Total plant dry mass (g)	Tracer recovered (mg/tot. pl. mass)	% recovery	Plant uptake (mg /g)
A103	BROMIDE	0.31	0.08	2.80	0.26
A203	BROMIDE	0.30	0.062	2.17	0.21
A302	BROMIDE	0.30	0.09	3.15	0.30
A405	BROMIDE	0.15	0.03	1.05	0.20
Mean		0.27	0.07	2.29	0.24
Std. Dev		0.08	0.03	0.92	0.05
A106	2,6-DFBA	0.30	0.35	7.59	1.17
A204	2,6-DFBA	0.30	0.43	9.33	1.43
A306	2,6-DFBA	0.24	0.31	6.72	1.29
A404	2,6-DFBA	0.28	0.50	10.85	1.79
Mean		0.28	0.40	8.62	1.42
Std. Dev		0.03	0.08	1.84	0.27
A105	PFBA	0.39	5.85E-5	0.0015	1.5E-4
A202	PFBA	0.20	ND		
A305	PFBA	0.20	4.76E-3	0.119	0.0238
A403	PFBA	0.19	7.50E-3	0.19	0.039
Mean		0.25	0.0041	0.10	0.02
Std. Dev		0.10	0.0038	0.095	0.0196

Table 13. Results of the analysis of barley plant extracts

Sample name	Treatment	Total plant dry mass (g)	Tracer recovered (mg/tot. pl. mass)	% recovery	Plant uptake (mg /g)
B103	BROMIDE	0.310	0.100	3.59	0.32
B201	BROMIDE	0.326	0.130	4.67	0.40
B303	BROMIDE	0.346	0.262	9.41	0.76
B406	BROMIDE	0.190	0.072	2.59	0.38
Mean		0.29	0.14	5.10	0.47
Std.Devn		0.07	0.08	3.0	0.2
B106	2,6-DFBA	0.492	1.09	27.25	2.22
B206	2,6-DFBA	0.416	0.97	24.25	2.33
B304	2,6-DFBA	0.424	0.89	22.25	2.10
B402	2,6-DFBA	0.218	0.55	13.75	2.52
Mean		0.39	0.88	21.88	2.29
Std.Devn		0.12	0.23	5.8	0.18
B105	PFBA	0.419	0.084	2.1	0.2
B207	PFBA	0.338	0.065	1.63	0.19
B306	PFBA	0.446	0.087	2.18	0.20
B401	PFBA	0.258	0.0325	0.81	0.13
Mean		0.37	0.07	1.68	0.18
Std.Devn		0.09	0.03	0.63	0.03

Table 14. Results of the analysis of canola plant extracts.

Sample name	Treatment	Total plant dry mass (g)	Tracer recovered (mg/tot. pl. mass)	% recoverey	Plant uptake (mg /g)
C103	BROMIDE	2.209	1.42	50.99	0.64
C203	BROMIDE	1.85	1.42	50.99	0.78
C302	BROMIDE	1.55	1.52	54.58	0.98
C404	BROMIDE	1.74	1.78	63.91	1.023
Mean		1.84	1.54	55.12	0.86
Std. Dev		0.28	0.17	6.10	0.18
C106	2,6-DFBA	2.59	2.165	54.12	0.84
C206	2,6-DFBA	2.58	1.73	43.25	0.67
C304	2,6-DFBA	2.60	2.12	53	0.82
C407	2,6-DFBA	3.00	1.76	44	0.60
Mean		2.69	1.94	48.60	0.73
Std. Dev		0.21	0.23	5.76	0.12
C105	PFBA	2.33	1.35	33.75	0.60
C202	PFBA	1.74	0.785	19.63	0.45
C303	PFBA	2.20	0.7365	18.41	0.33
C403	PFBA	2.68	0.125	3.13	0.05
Mean		2.24	0.75	18.75	0.36
Std. Dev		0.39	0.50	12.52	0.23

values for 2,6-DFBA and PFBA were 2.96 and 3.38 respectively. The higher the log K_{ow} values of a compound the higher the chance will be for its uptake by plants (due to the preference for the like medium over the aqueous medium). The higher log K_{ow} value of PFBA suggests that it should be taken up more than the 2,6-DFBA. However, there is another factor that also controls the uptake and that is the pK_a. The pH of the medium should be at least two units below the pK_a of any organic acid, for the major portion of that acid to exist in protonated form. Then it will show higher rate of partition into the organic phase. PFBA has the lowest pK_a (2.7) of all the fluorobenzoates. Even though PFBA has relatively higher log K_{ow} value, its low pK_a results in a smaller fraction exisiting in the protonated form at any given pH, thus resulting in its lower uptake by plants. Based on the pK_a values of 2,6-DFBA(3.0) and 3,4-DFBA(3.7), the latter compound should show relatively higher uptake by plants. The results of the analysis of soil extracts of alfalfa, canola and barley show the least recovery of 3,4-DFBA among the tracers. This may be due to its higher uptake by plants.

Several workers studying the phenolic acids absorption and their effect upon the ion absorption by plants observed that the lower the pH of nutrient medium the greater the inhibitory effect on ion absorption by plant roots (Glass 1973, 1974, 1975; Harper and Balke 1981). An increase in the rates of uptake of salicylic acid, ferrulic acid and *p*-hydroxy benzoic acid as the pH of nutrient medium was lowered, was reported by Harper and Balke (1981) and Shann and Blum (1987).

Tables 15, 16 and 17 show the mass balance achieved for the three plants for three tracers (Br, 2,6-DFBA, and PFBA). PFBA showed the minimum mass balance achieved amongst the three compounds. Average mass balance achieved for PFBA was 72%, 70%, and 70% for alfalfa, barley, and canola plants respectively. An average mass balance of 92%, 81%, and 79% was achieved in the case of 2,6-DFBA for the alfalfa, barley and canola plants.

100% mass balances were not achieved probably due to the metabolic transformation of the fluorobenzoates within the plant tissue. Table 18 shows the average mass of plant material for four replicates within each tracer treatment, followed by the mass balance achieved, and number of days the plants were allowed to grow further after the application of tracer. There is an obvious and direct relation between the plant mass, number of days of plant growth after the tracer application, and the amount of missing mass of tracer. This suggests that metabolic transformation within the plant tissue may be a possible answer for the missing mass.

Table 19 shows a comparison of the recoveries and mass balance obtained for the 2,6-DFBA, by liquid scintillation counting and HPLC, for the three plant samples. A t-test was used to check if any significant differences exist between the means of percent recoveries obtained by liquid scintillation counting, oxidation and HPLC. The resultant t-values were below the t-critical values. This indicates that comparable results were obtained from the two methods.

Tables 20, 21 and 22 show the effect of these tracers on the growth of the three plants. This was done by comparing the relative dry weights of the plants treated with

Table 15. Mass balance results of the alfalfa samples.

Sample name	Treatment	Tracer amount from soil (mg)	Tracer amount from plant (mg)	Total (mg)	Amount applied (mg)	% recovery
A103	BROMIDE	2.65	0.08	2.73	2.86	95.45
A203	BROMIDE	2.42	0.062	2.48	2.86	86.78
A302	BROMIDE	2.73	0.09	2.82	2.86	98.60
A405	BROMIDE	2.55	0.03	2.58	2.86	90.21
Mean		2.59	0.07	2.65		92.76
Std. Dev		0.13	0.03	0.15		5.28
A106	2,6-DFBA	4.23	0.35	4.58	4.61	99.35
A204	2,6-DFBA	3.43	0.43	3.86	4.61	83.73
A306	2,6-DFBA	4.06	0.31	4.37	4.61	94.79
A404	2,6-DFBA	3.60	0.50	4.10	4.61	88.94
Mean		3.83	0.40	4.23		91.70
Std. Dev		0.38	0.08	0.31		6.81
A105	PFBA	2.808	5.85E-5	2.808	4	70.2
A202	PFBA	2.538	ND	2.538	4	63.45
A305	PFBA	2.742	4.76E-3	2.742	4	68.55
A403	PFBA	3.408	7.5E-3	3.408	4	85.20
Mean		2.87	0.0041	2.88		71.85
Std. Dev		0.38	0.0038	0.37		9.35

Table 16. Mass balance results of the barley samples.

Sample name	Treatment	Tracer amount from soil (mg)	Tracer amount from plant (mg)	Total (mg)	Amount applied (mg)	% recovery
B103	BROMIDE	2.99	0.100	3.09	2.785	110.95
B201	BROMIDE	2.73	0.130	2.86	2.785	102.69
B303	BROMIDE	2.81	0.262	3.07	2.785	110.23
B406	BROMIDE	3.63	0.072	3.70	2.785	132.85
Mean		3.04	0.14	3.18		114.18
Std. Dev		0.41	0.08	0.36		13.00
B106	2,6-DFBA	2.25	1.09	3.34	4	83.5
B206	2,6-DFBA	2.19	0.97	3.16	4	79
B304	2,6-DFBA	2.32	0.89	3.21	4	80.25
B402	2,6-DFBA	2.62	0.55	3.17	4	79.25
Mean		2.35	0.88	3.22		80.50
Std. Dev		0.19	0.23	0.08		2.07
B105	PFBA	2.36	0.084	2.44	4	61.10
B207	PFBA	2.79	0.065	2.86	4	71.38
B306	PFBA	2.68	0.087	2.77	4	69.18
B401	PFBA	3.13	0.0325	3.16	4	79.06
Mean		2.74	0.067	2.81		70.18
Std. Dev		0.32	0.02	0.30		7.39

Table 17. Mass balance results of the canola samples.

Sample name	Treatment	Tracer amount from soil (mg)	Tracer amount from plant (mg)	Total (mg)	Amount applied (mg)	% recovery
C103	BROMIDE	1.46	1.42	2.88	2.785	103.41
C203	BROMIDE	1.29	1.42	2.71	2.785	97.31
C302	BROMIDE	1.34	1.52	2.86	2.785	102.70
C404	BROMIDE	1.27	1.78	3.05	2.785	109.52
Mean		1.34	1.54	2.88		103.23
Std. Dev		0.09	0.17	0.14		5.00
C106	2,6-DFBA	1.34	2.165	3.51	4	87.75
C206	2,6-DFBA	1.36	1.73	3.09	4	77.25
C304	2,6-DFBA	0.83	2.12	2.95	4	73.75
C407	2,6-DFBA	1.31	1.76	3.07	4	76.75
Mean		1.21	1.94	3.16		78.88
Std. Dev		0.25	0.23	0.25		6.12
C105	PFBA	1.59	1.35	2.94	4	73.50
C202	PFBA	1.86	0.785	2.645	4	66.13
C303	PFBA	2.26	0.7365	3.00	4	75
C403	PFBA	2.41	0.125	2.535	4	63.38
Mean		2.03	0.75	2.78		69.50
Std. Dev		0.37	0.5	0.22		5.63

Table 18. Table showing a relationship between mass balance missing and mass of plant material and number of days of growth after application. (A = alfalfa, B = barley, C = canola)

Sample name	Average plant dry mass (g)	Average % mass balance	Number of days of growth after application
APFBA	0.245	72	7
A2,6-DFBA	0.28	92	7
BPFBA	0.365	70.18	11
B2,6-DFBA	0.3875	80.50	11
CPFBA	2.24	69.50	13
C2,6-DFBA	2.70	79	13

Table 19. Comparision of recoveries and mass balances obtained for the 2,6-DFBA samples using liquid scintillation counting and HPLC.

Sample name	% recovery from plant			% recovery from soil		% total recovery	
	A.lsc*	O.lsc	HPLC	HPLC	A.lsc	HPLC	A.l.sc
A106	9	10.7	7.59	91.8	80.1	99.4	89.1
A204	13	14.2	9.33	74.4	68.6	83.7	81.6
A306	8	5.4	6.72	88.1	75.7	95.4	83.7
A404	15.5	10.3	10.9	78.1	68.8	88.9	84.3
Mean	11.4	10.2	8.62	83.1	73.3	91.9	84.7
Std. Devn	3.5	3.6	1.8	8.2	5.6	6.9	3.2
t values			0.75				1.88
B106	22	29	27.3	56.3	55.0	83.5	77.0
B204	25	30	24.3	54.8	50.5	79	75.5
B304	21	25	22.3	58	55.2	80.3	76.2
B402	15	18	13.8	65.5	62.1	79.3	77.1
Mean	20.8	25.5	21.9	58.6	55.7	80.5	76.5
Std. Devn	4.2	5.4	5.8	4.8	4.8	2.1	0.8
t values#			0.91				3.71+
C106	48	61	54.1	33.5	29.9	87.6	77.9
C206	43	39	43.3	34	31.1	77.3	74.1
C304	45	53	53	20.8	19.4	73.8	64.4
C407	44	45	44	32.8	27.9	76.8	71.9
Mean	45	49.5	48.6	30.3	27.1	78.9	72.1
Std. Devn	2.2	9.6	5.8	6.4	5.3	6.1	5.7
t values			0.16				1.63

*A.lsc = Liquid Scintillation counting of aqueous extracts; O.lsc = Oxidation of plant material

+ T value at 90% confidence level

t values are for comparision of O.lsc and HPLC in case of recovery from plant extracts and for A.lsc and HPLC in case of total recovery

Table 20. Effect of various tracers on alfalfa plant growth as compared to controls

Sample name	Treatment	Total plant mass (g)	Average mass (g) (std.devn)	Index
A101	CONTROL	0.38		
A206	CONTROL	0.41		
A301	CONTROL	0.33		
A401	CONTROL	0.32	0.36 (0.042)	100
A103	BROMIDE	0.31		
A203	BROMIDE	0.30		
A302	BROMIDE	0.30		
A405	BROMIDE	0.15	0.27 (0.077)	75
A104	3,4-DFBA	0.38		
A201	3,4-DFBA	0.36		
A307	3,4-DFBA	0.30		
A406	3,4-DFBA	0.25	0.3225 (0.059)	90
A105	PFBA	0.39		
A202	PFBA	0.20		
A305	PFBA	0.20		
A403	PFBA	0.19	0.245 (0.097)	68
A106	2,6-DFBA	0.30		
A204	2,6-DFBA	0.30		
A306	2,6-DFBA	0.24		
A404	2,6-DFBA	0.28	0.28 (0.028)	78

Table 21. Effect of various tracers on barley plant growth as compared to controls

Sample name	Treatment	Total plant dry mass (g)	Average mass (g) (std. devn)	Index
B102	CONTROL	0.426		
B206	CONTROL	0.292		
B307	CONTROL	0.186		
B405	CONTROL	0.271		
B101	CONTROL	0.321		
B203	CONTROL	0.331		
B302	CONTROL	0.341		
B404	CONTROL	0.281	0.306 (0.069)	100
B103	BROMIDE	0.310		
B201	BROMIDE	0.326		
B303	BROMIDE	0.346		
B406	BROMIDE	0.190	0.293 (0.070)	96
B104	3,4-DFBA	0.412		
B205	3,4-DFBA	0.249		
B301	3,4-DFBA	0.281		
B403	3,4-DFBA	0.217	0.290 (0.086)	95
B105	PFBA	0.419		
B207	PFBA	0.338		
B306	PFBA	0.446		
B401	PFBA	0.258	0.365 (0.085)	119
B106	2,6-DFBA	0.492		
B204	2,6-DFBA	0.416		
B304	2,6-DFBA	0.424		
B402	2,6-DFBA	0.218	0.3875 (0.12)	126

Table 22. Effect of various tracers on canola plant growth as compared to controls

Sample name	Treatment	Total plant dry mass (g)	Average mass (g) (std. devn)	Index
C102	CONTROL	1.67		
C205	CONTROL	1.82		
C301	CONTROL	2.18		
C306	CONTROL	1.75		
C401	CONTROL	1.61	1.806 (0.22)	100
C103	BROMIDE	2.209		
C203	BROMIDE	1.85		
C302	BROMIDE	1.55		
C404	BROMIDE	1.74	1.84 (0.28)	102
C104	3,4-DFBA	1.45		
C201	3,4-DFBA	1.15		
C305	3,4-DFBA	1.89		
C406	3,4-DFBA	2.98	1.86 (0.80)	103
C105	PFBA	2.33		
C202	PFBA	1.74		
C303	PFBA	2.20		
C403	PFBA	2.68	2.24 (0.39)	124
C106	2,6-DFBA	2.59		
C206	2,6-DFBA	2.58		
C304	2,6-DFBA	2.60		
C407	2,6-DFBA	3.00	2.70 (0.21)	150

the four compounds to the dry weights of control plants. No major retardation effects were noticed on the growth of the barley and canola plants. Alfalfa plants treated with bromide, 2,6-DFBA and PFBA showed relatively lower dry weights. The lack of any toxic effect on barley and canola plants may be due to a short time of exposure or they may be more resistant at higher levels of fluorobenzoates.

CONCLUSIONS AND RECOMMENDATIONS

Green house experiments were conducted to determine the plant uptake of fluorobenzoates used as soil and groundwater tracers. Three fluorobenzoates, PFBA, 2,6-DFBA, and 3,4-DFBA were studied for their uptake by alfalfa, barley and canola plants. A method was developed for the analysis of fluorobenzoates in plant material. The analytical method gave consistent recoveries from spiked plant extracts and worked well for the analyses of PFBA and 2,6-DFBA in plants. The results for 3,4-DFBA in plant extracts were not available, due to its low retention time which resulted in poor resolution of the 3,4-DFBA peak from the interference or solvent peak. Based on the recoveries from the soil 2,6-DFBA showed relatively higher uptake than PFBA, in all the three plant species tested. Canola plants showed maximum uptake of all the three compounds. 100% mass balances were not achieved, probably due to the metabolic transformation within the plants.

These experiments were done only for three crop plant species. If these tracers need to be used in situations involving any other plant species, preliminary studies need to be done for their toxic effects.

These studies were conducted in pots which were sealed to prevent any drainage, so these studies reflect a worst-case scenario. In real field situations there is a great possibility for the tracers to leach away from the surface and root zone. It will be very interesting to do a field-scale experiment which will give plant uptake results under more real-life conditions. The effects of the fluorobenzoates on the bacteria that are symbiotic to plants needs to be studied.

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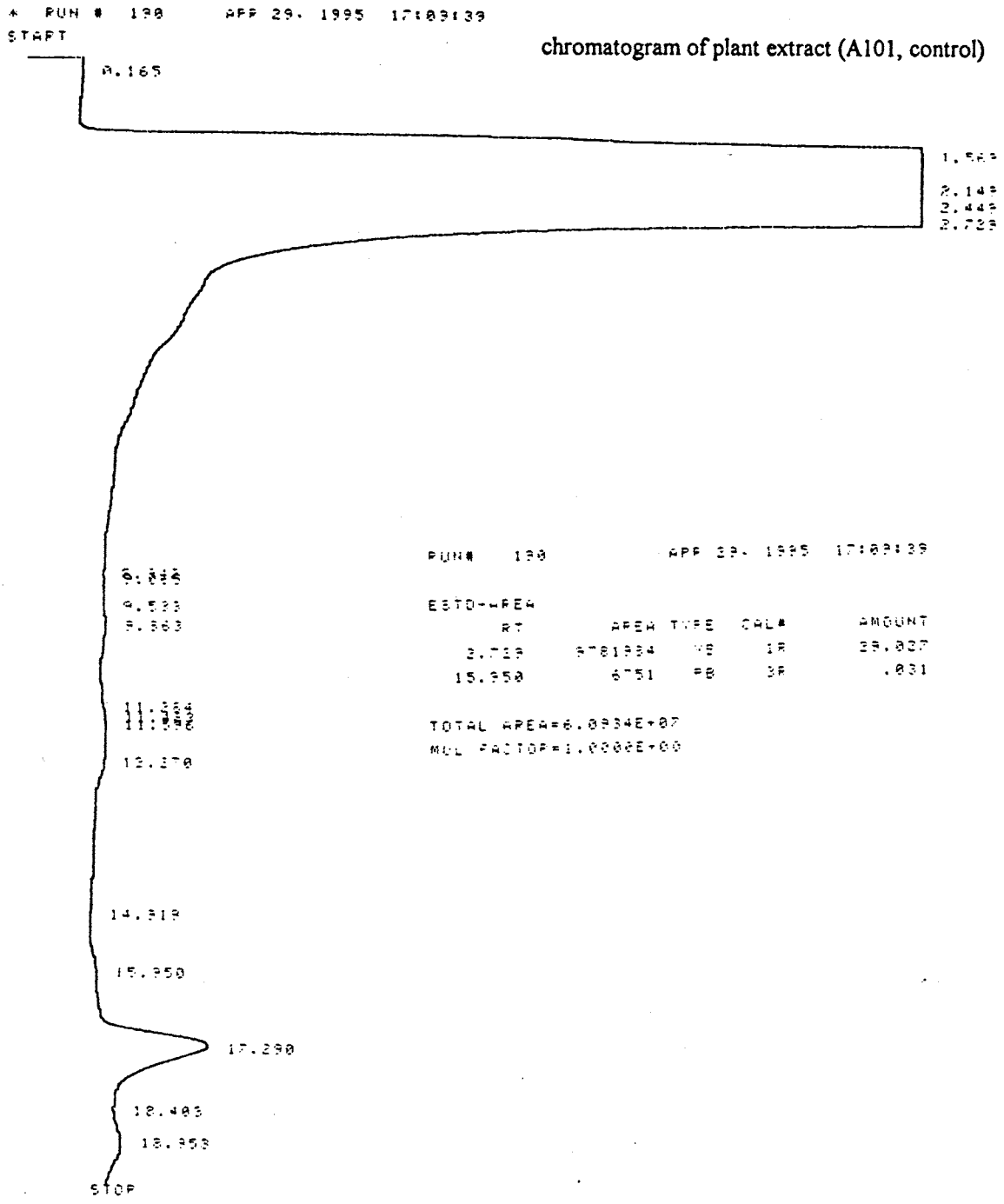
APPENDIX A

CHROMATOGRAMS OF PLANT EXTRACTS

The following pages include the copies of chromatograms of plant extracts. For chromatographic conditions please refer to the Materials and Methods section. Even though the analysis was performed in duplicate for each treatment, only one chromatogram for each tracer treatment was included in this Appendix. The chromatograms are labeled with the first letter of the plant name, followed by sample number and tracer treatment. Controls are chromatograms of plant extracts that were not treated with any tracer.

chromatogram of plant extract (A101, control)



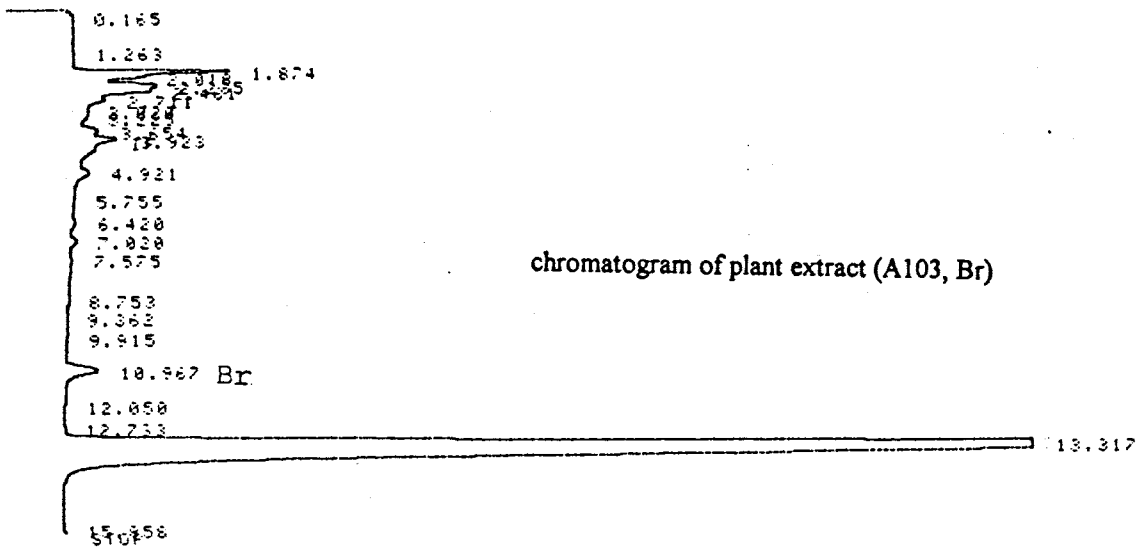
RUN# 190 APR 29, 1995 17:09:39

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
2.719	6781934	VB	1R	99.827
15.950	6751	PB	3R	.031

TOTAL AREA=6.0934E+07
MUL FACTOR=1.0000E+00

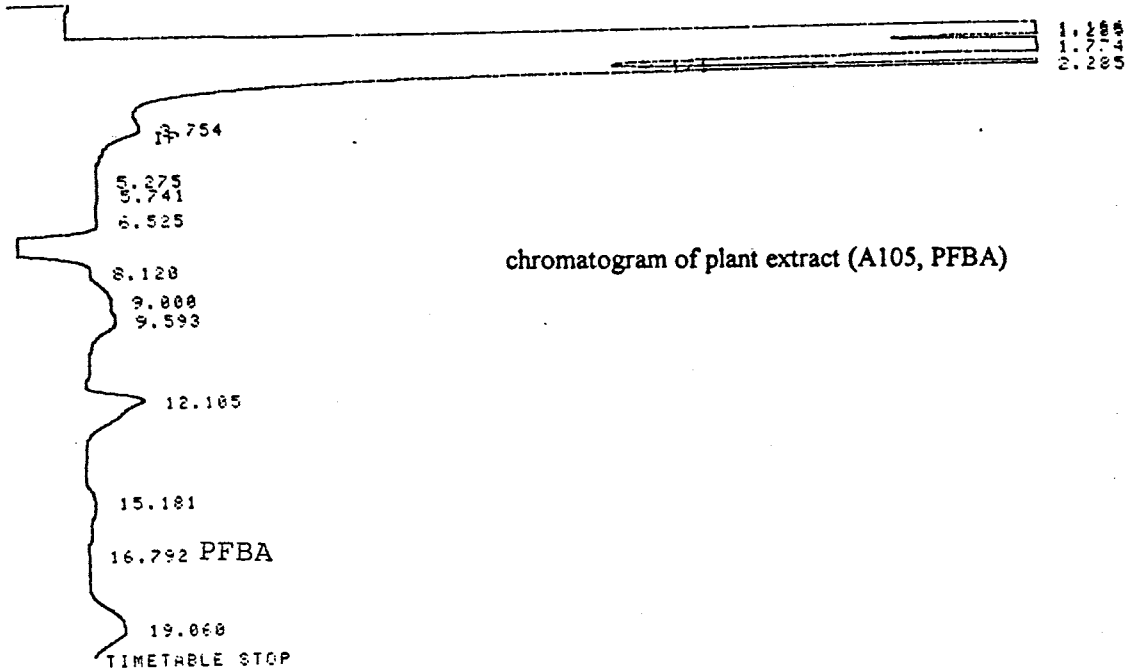
```
*  RUN # 1557      NOV 29, 1994   19:28:34
START
```



chromatogram of plant extract (A103, Br)

```
RUN#  1557           NOV 29, 1994  19:28:34

ESTD-AREA
      RT        AREA TYPE  CAL#       AMOUNT
  10.967      346787   BB    1R         .799

TOTAL AREA=2.2767E+07
MUL FACTOR=1.0000E+00



CALIBRATION

ESTD
REF % RTW:    5.000  NON-REF % RTW:    5.000

LEVEL: 1              RECALIBRATIONS: 1

CAL#      RT      LV     AMT       AMT/AREA
  1F   11.312     1 5.0000E+00  3.3029E-06

CAL#      NAME
  1  BP

CALIBRATION OPTIONS
```

* RUN # 1493 NOV 7, 1994 13:39:02
START



chromatogram of plant extract (A105, PFBA)

RUN# 1493 NOV 7, 1994 13:39:02

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
16.792	197	FB	1R	.001

TOTAL AREA=8.277SE+07
MUL FACTOR=1.0000E-00

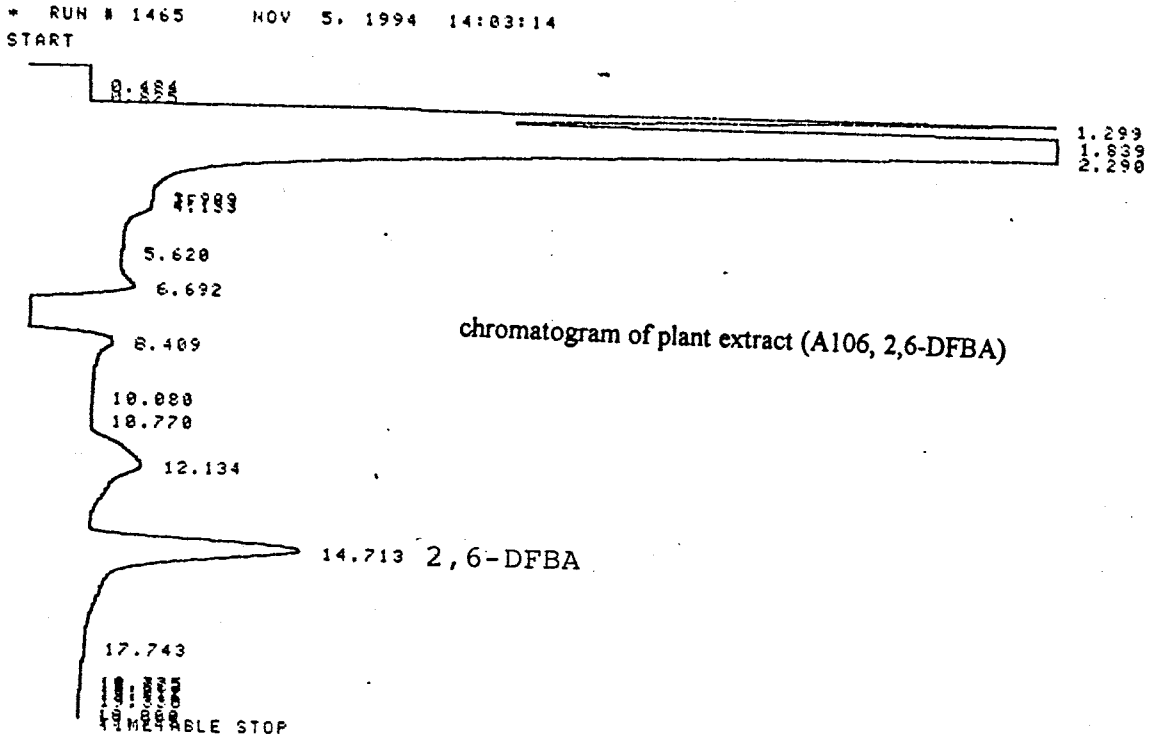
CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	16.218	1	5.0000E+00	6.6922E-06

CAL#	NAME
1	PFBA



chromatogram of plant extract (A106, 2,6-DFBA)

RUN# 1465 NOV 5, 1994 14:03:14

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
14.713	4916154	BB	1R	21.575

TOTAL AREA=8.5780E+07
MUL FACTOR=1.0000E+00

CALIBRATION

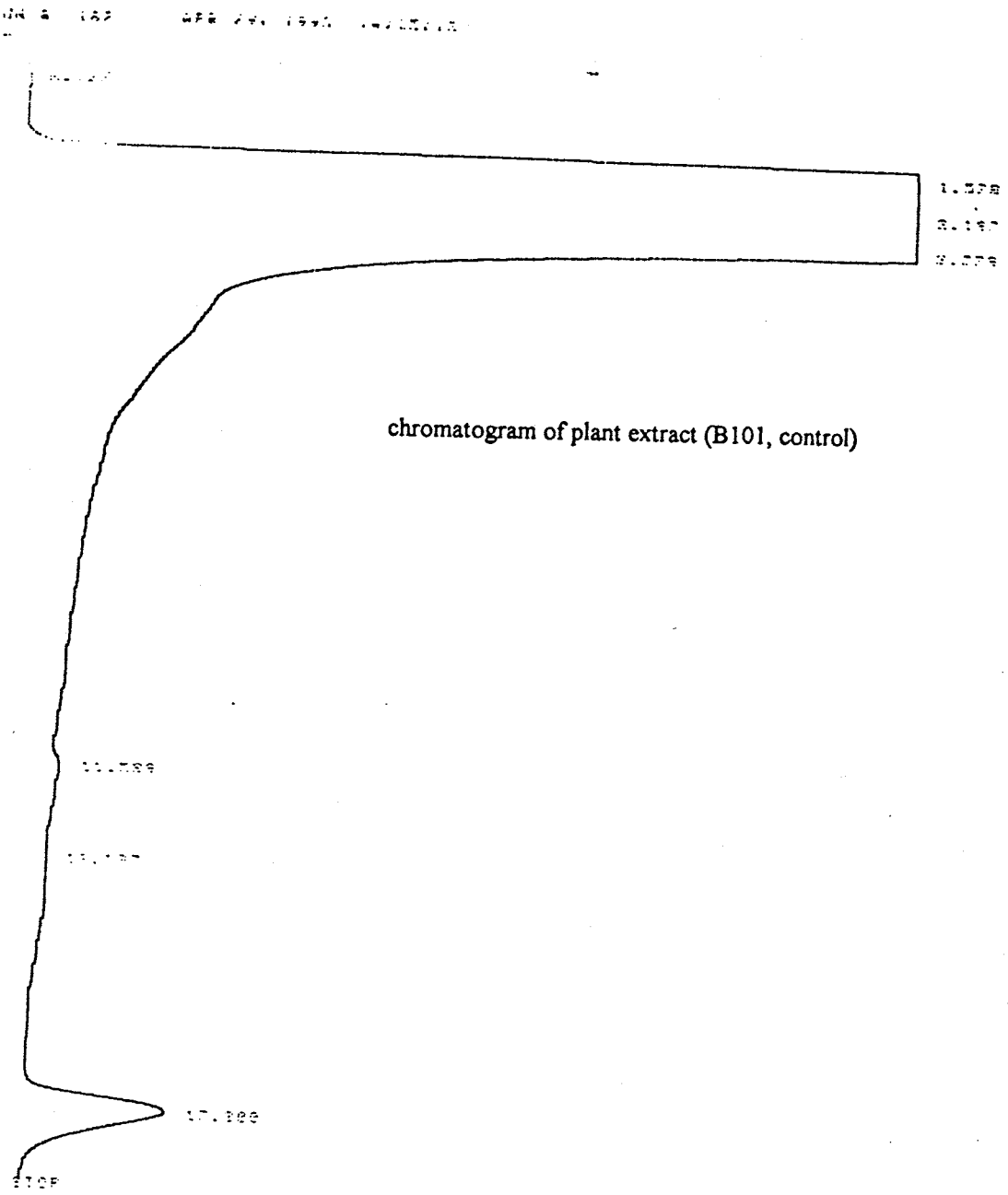
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

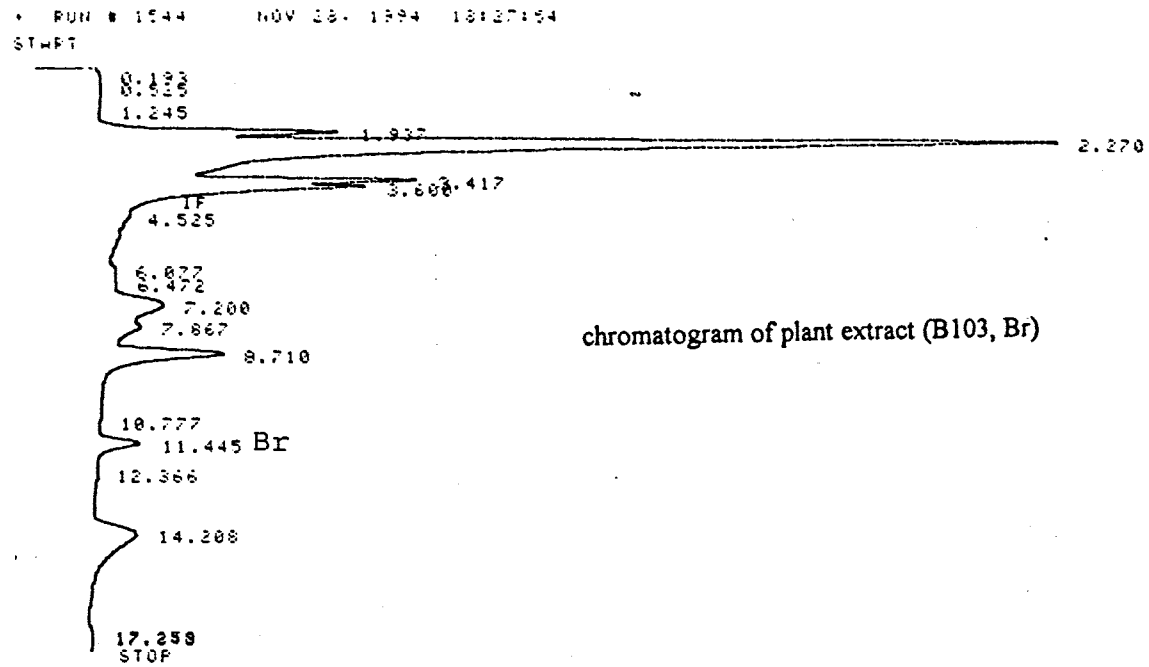
CAL#	RT	LV	AMT	AMT/AREA
1R	14.718	1	5.0000E+00	4.3866E-06

CAL#	NAME
1	2,6-DFBA

CALIBRATION OPTIONS



chromatogram of plant extract (B101, control)



chromatogram of plant extract (B103, Br)

RUN# 1544 NOV 28, 1994 18:27:54

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
11.445	443601	BB	1R	1.022

TOTAL AREA=2.2139E+07
MUL FACTOR=1.0000E+00

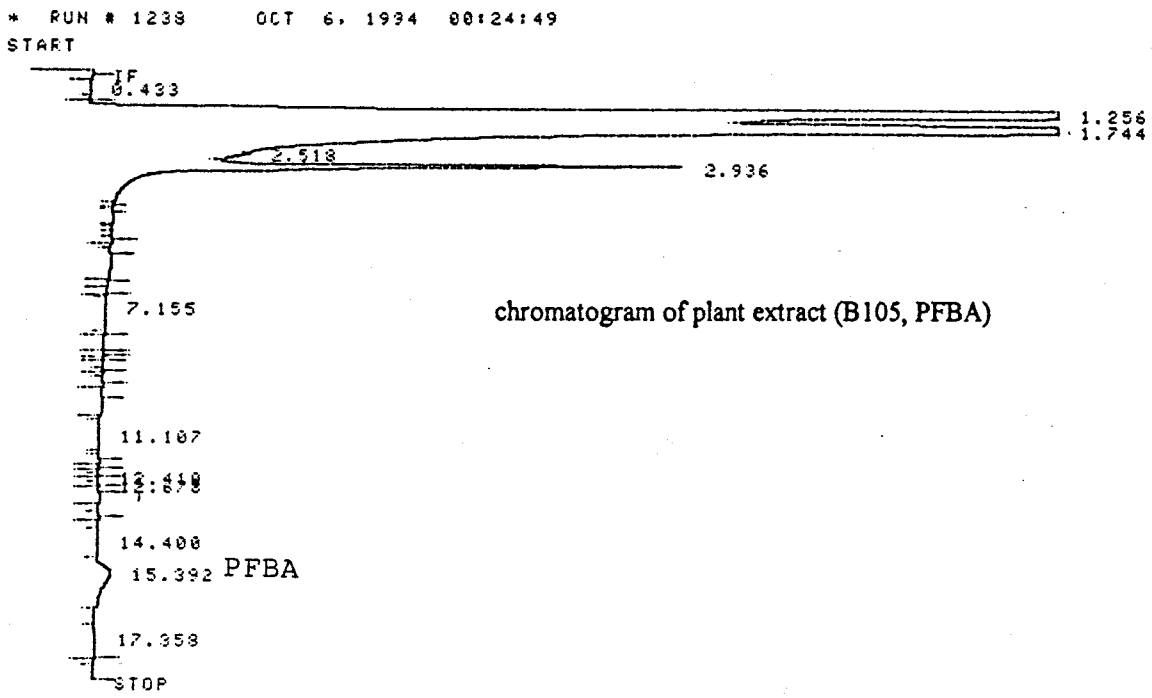
CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	11.443	1	5.0000E+00	2.3029E-06

CAL#	NAME
1	BR



chromatogram of plant extract (B105, PFBA)

RUN# 1238 OCT 6, 1994 00:24:49

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
15.392	323434	VV	1R	2.088

TOTAL AREA=5.2065E+07
MUL FACTOR=1.0000E+00

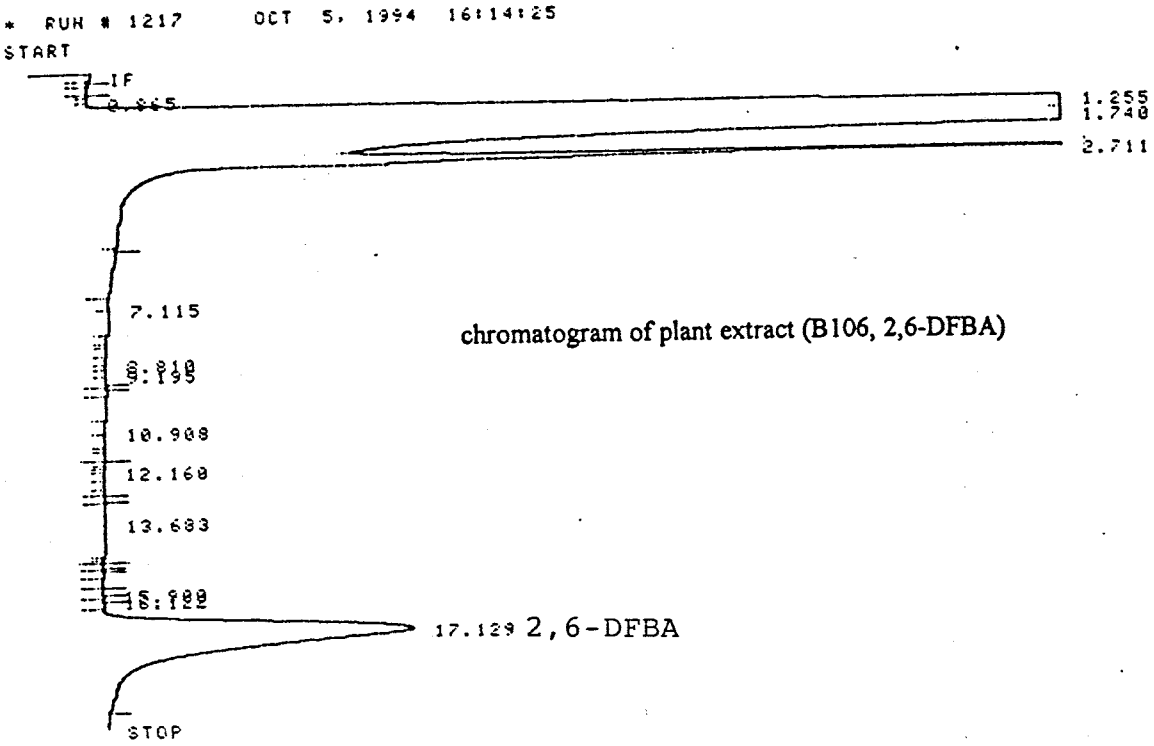
CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	15.437	1	5.0000E+00	6.4561E-06

CAL#	NAME
1	PFBA



chromatogram of plant extract (B106, 2,6-DFBA)

RUN# 1217 OCT 5, 1994 16:14:25

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
17.139	8236979	BB	1R	36.645

TOTAL AREA=9.7979E+07
MUL FACTOR=1.0000E+00

CALIBRATION

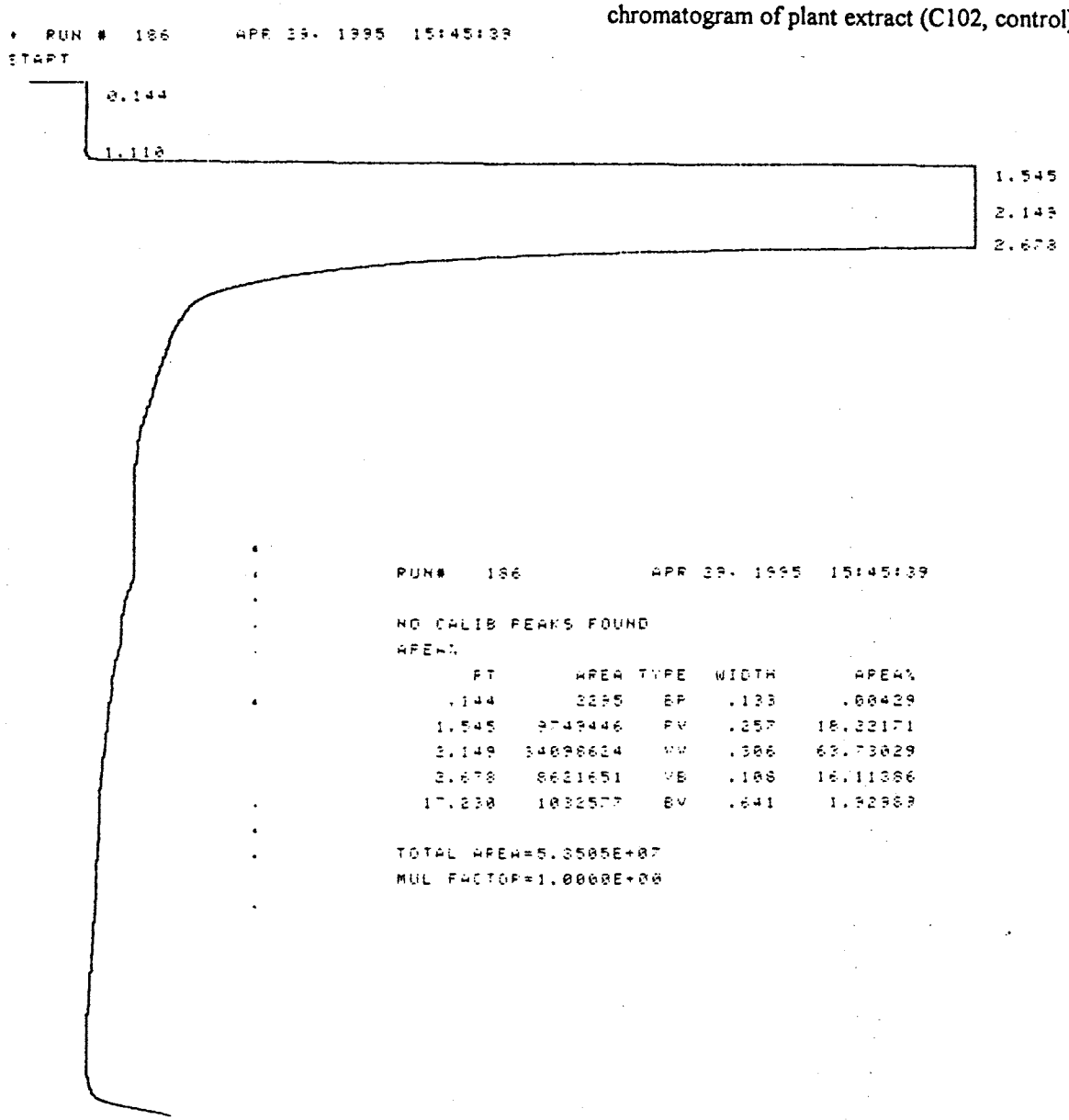
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	17.294	1	2.0000E+01	4.4489E-06

CAL#	NAME
1	2,6-DFBA

chromatogram of plant extract (C102, control)



RUN# 186 APR 29, 1995 15:45:39

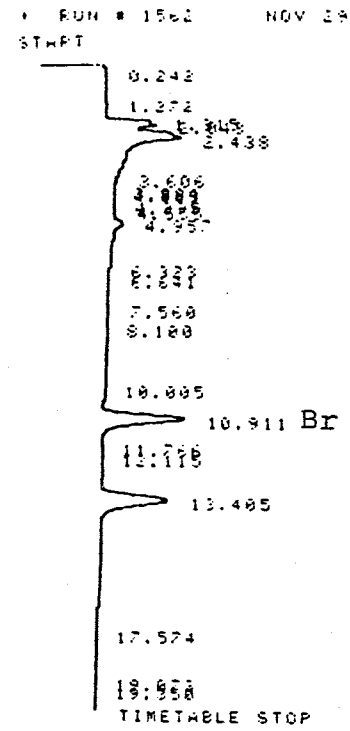
NO CALIB PEAKS FOUND

AREA%

PT	AREA	TYPE	WIDTH	AREA%
.144	2295	BP	.133	.00429
1.545	9749446	PV	.257	18.22171
2.149	34098634	VV	.306	63.73029
2.678	8621651	VB	.108	16.11386
17.230	1032577	BV	.641	1.92969

TOTAL AREA=5.3505E+07

MUL FACTOR=1.0000E+00



chromatogram of plant extract (C103, Br)

RUN# 1562 NOV 29, 1994 21:28:52

ESTD-AREA

RT	AREA	TYPE	CHL#	AMOUNT
10.911	837906	BB	1R	1.930

TOTAL AREA=3502214
MUL FACTOR=1.0000E+00

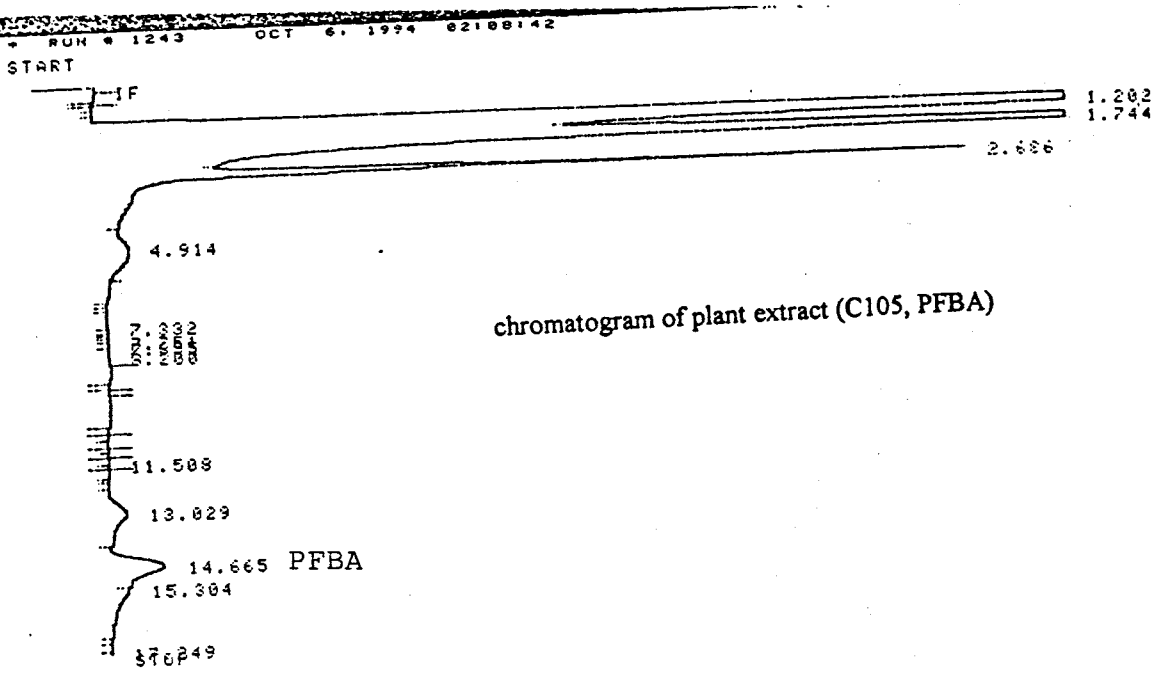
CALIBRATION

ESTD
REF % RTN: 5.000 NON-REF % RTN: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CHL#	RT	LV	AMT	AMT/AREA
1R	11.033	1	5.0000E+00	2.3029E-06

CHL#	NAME
1	BR



chromatogram of plant extract (C105, PFBA)

RUN# 1243 OCT 6, 1994 02:08:42

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
14.665	1069818	VV	1R	6.907

TOTAL AREA=4.2577E+07
MUL FACTOR=1.0000E+00

CALIBRATION

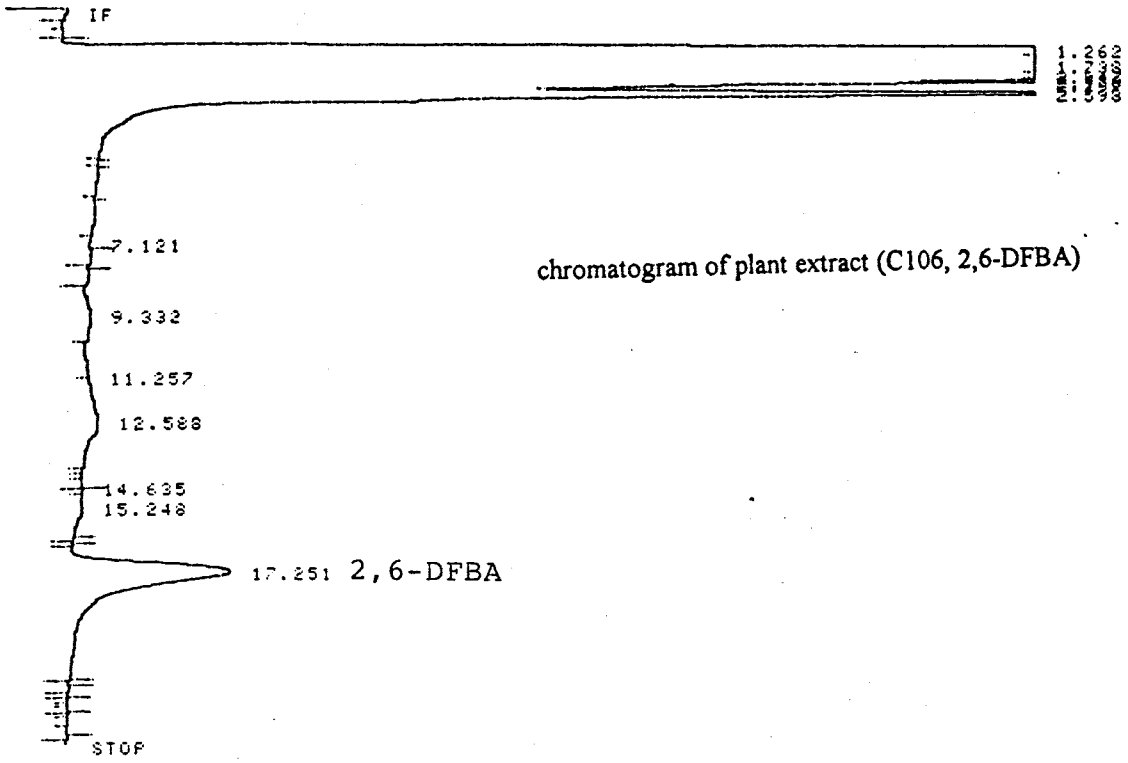
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	15.420	1	5.0000E+00	6.4561E-06

CAL#	NAME
1	PFBA

```
*  RUN # 1226      OCT  5, 1994  19:46:18
START
```



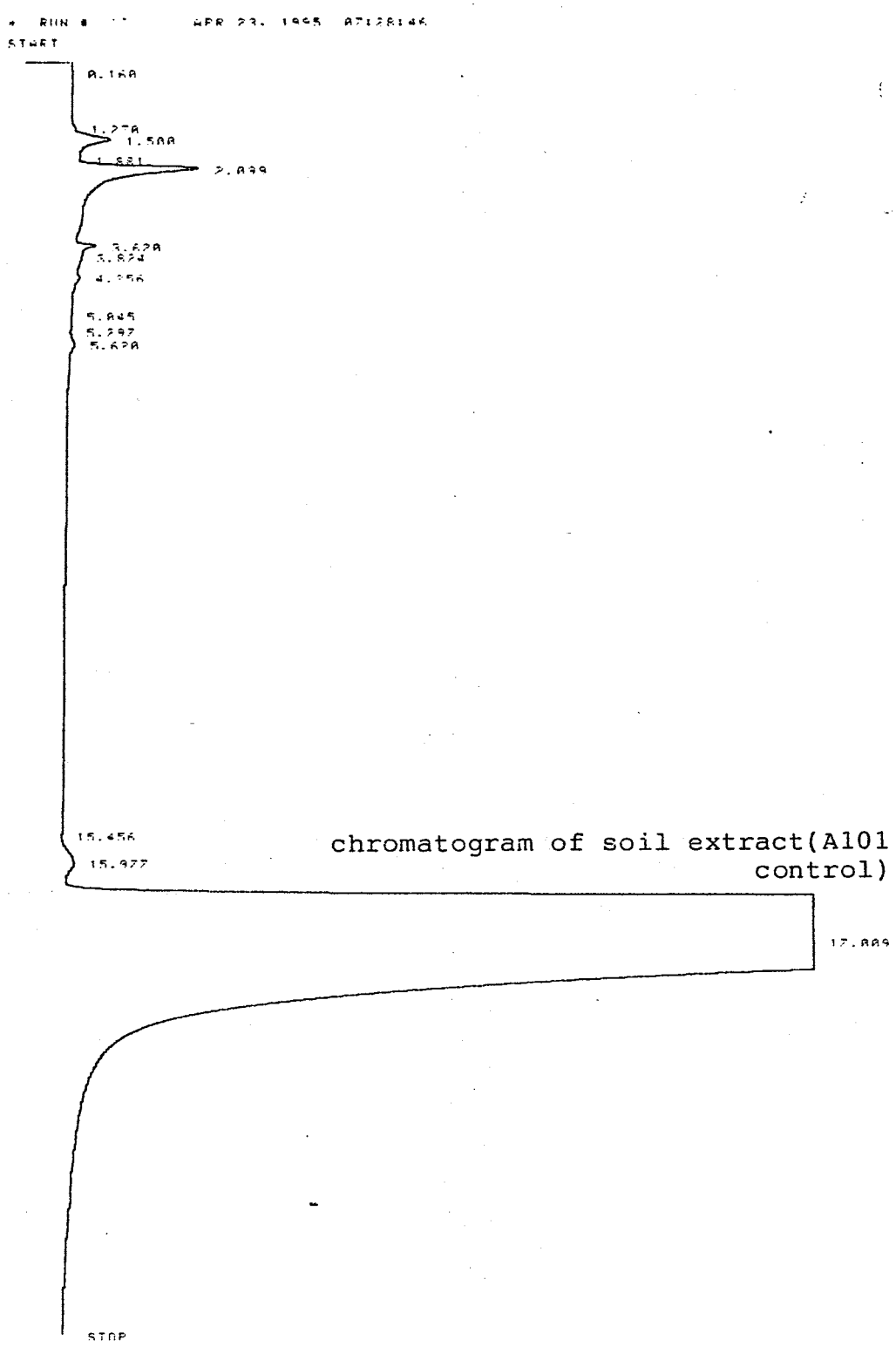
chromatogram of plant extract (C106, 2,6-DFBA)

```
RUN#  1226           OCT  5, 1994  19:46:18

ESTD-AREA
      RT        AREA TYPE  CAL#       AMOUNT
  17.251     4201779   BB     1R       18.693

TOTAL AREA=8.0151E+07
MUL FACTOR=1.0000E+00



CALIBRATION

ESTD
REF % RTW:    5.000  NON-REF % RTW:    5.000

LEVEL: 1             RECALIBRATIONS: 1

CAL#      RT,     LV     AMT       AMT/AREA
  1R   17.635     1 2.0000E+01  4.4489E-06

CAL#      NAME
  1  2,6-DFBA
```

APPENDIX B

CHROMATOGRAMS OF SOIL EXTRACTS

The following pages include the copies of chromatograms of soil extracts. For chromatographic conditions please refer to the materials and methods section. Even though the analysis was performed in duplicate for each treatment, only one chromatogram for each tracer treatment was included in this appendix. The chromatograms are labeled with the first letter of the plant name, followed by sample number and tracer treatment in parenthesis. Controls are chromatograms of soil extracts that were not treated with any tracer.



chromatogram of soil extract(A101 control)

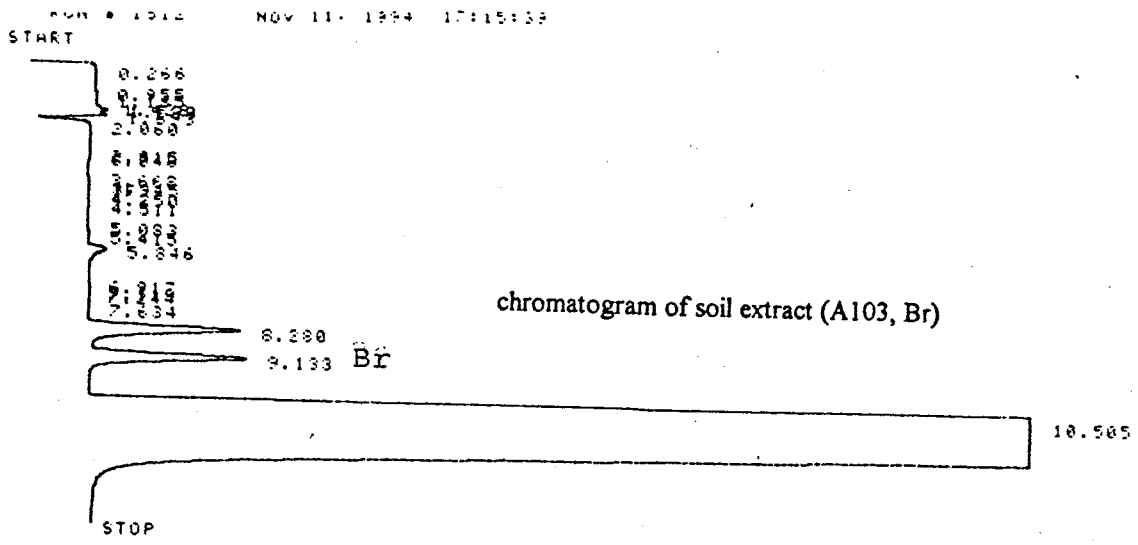
RUN# 171 APR 23, 1995 07:28:46

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
15.456	17	FB	2P	.000

TOTAL AREA=4.5719E+05

MUL FACTOR=1.0000E+00



chromatogram of soil extract (A103, Br)

RUN# 1512 NOV 11, 1994 17:15:39

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
9.133	1267296	BB	1R	4.078

TOTAL AREA=4.3649E+08
MUL FACTOR=1.0000E+00

CALIBRATION

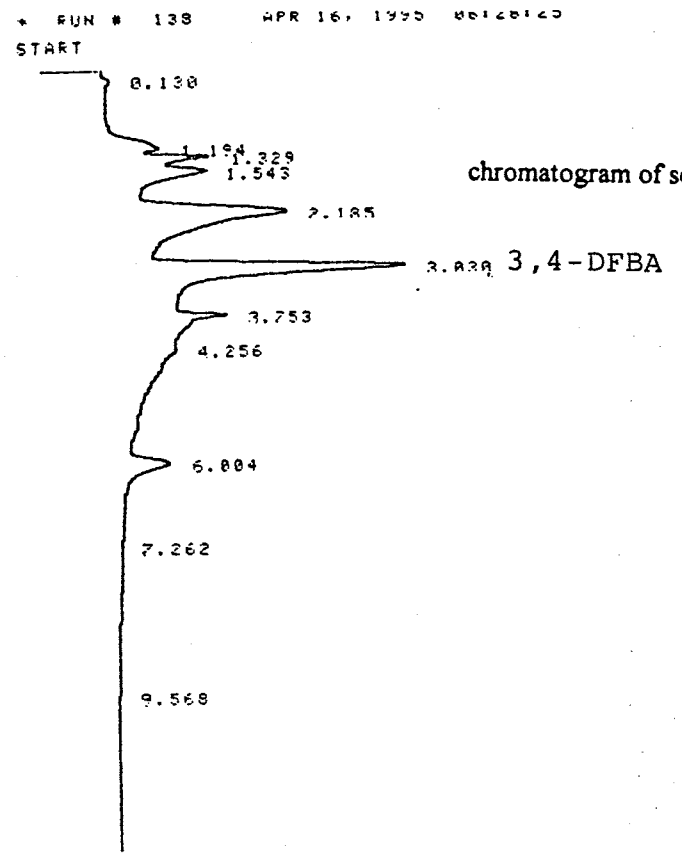
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT AREA
1R	9.217	1	5.0000E+00	3.3179E-06

CAL#	NAME
1	BROMIDE

CALIBRATION OPTIONS



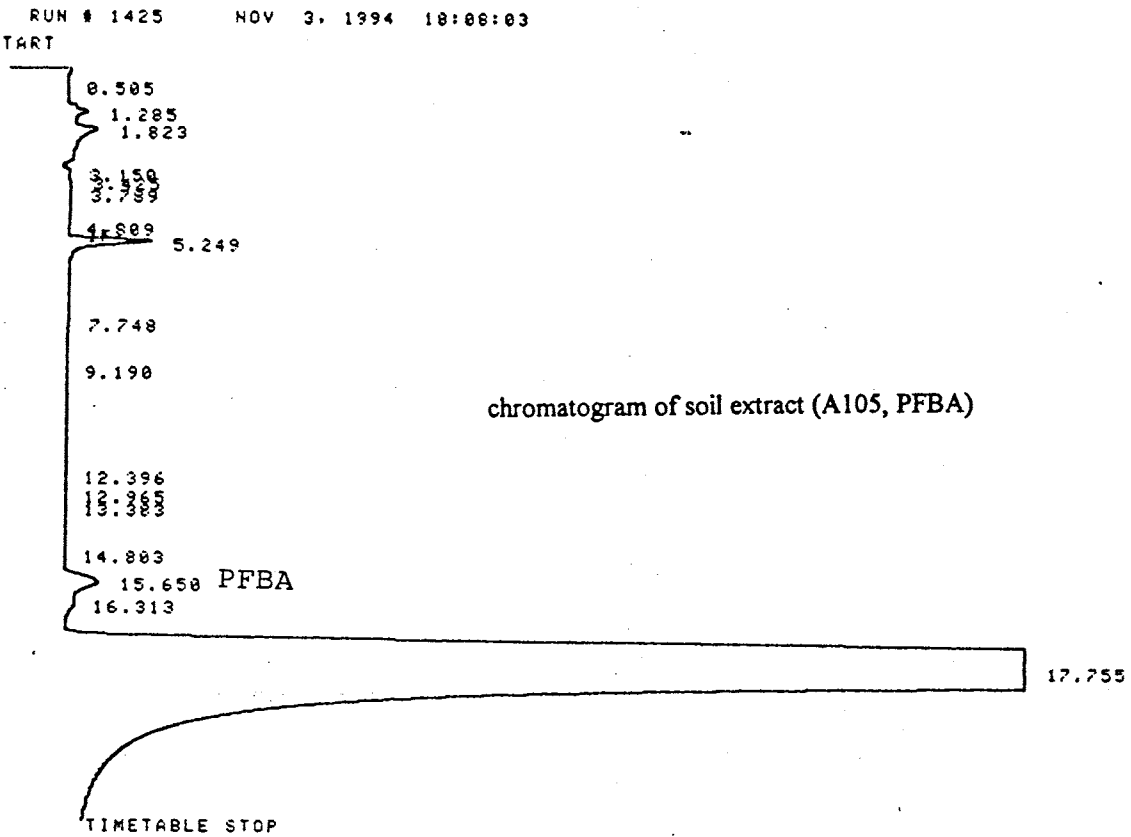
chromatogram of soil extract (A104, 3,4-DFBA)

RUN# 138 APR 16, 1995 06:28:25

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
3.030	798271	PB	1R	2.319
15.769	18391	VV	2R	.052
17.215	166873	VV	3R	.742

TOTAL AREA=4.5272E+08
MUL FACTOR=1.0000E+00



chromatogram of soil extract (A105, PFBA)

RUN# 1425 NOV 3, 1994 18:08:03

ESTD-HEIGHT

RT	HEIGHT	TYPE	CAL#	AMOUNT
15.650	19964	VH	1R	4.454

TOTAL HEIGHT=8.7675E+06
MUL FACTOR=1.0000E+00

CALIBRATION

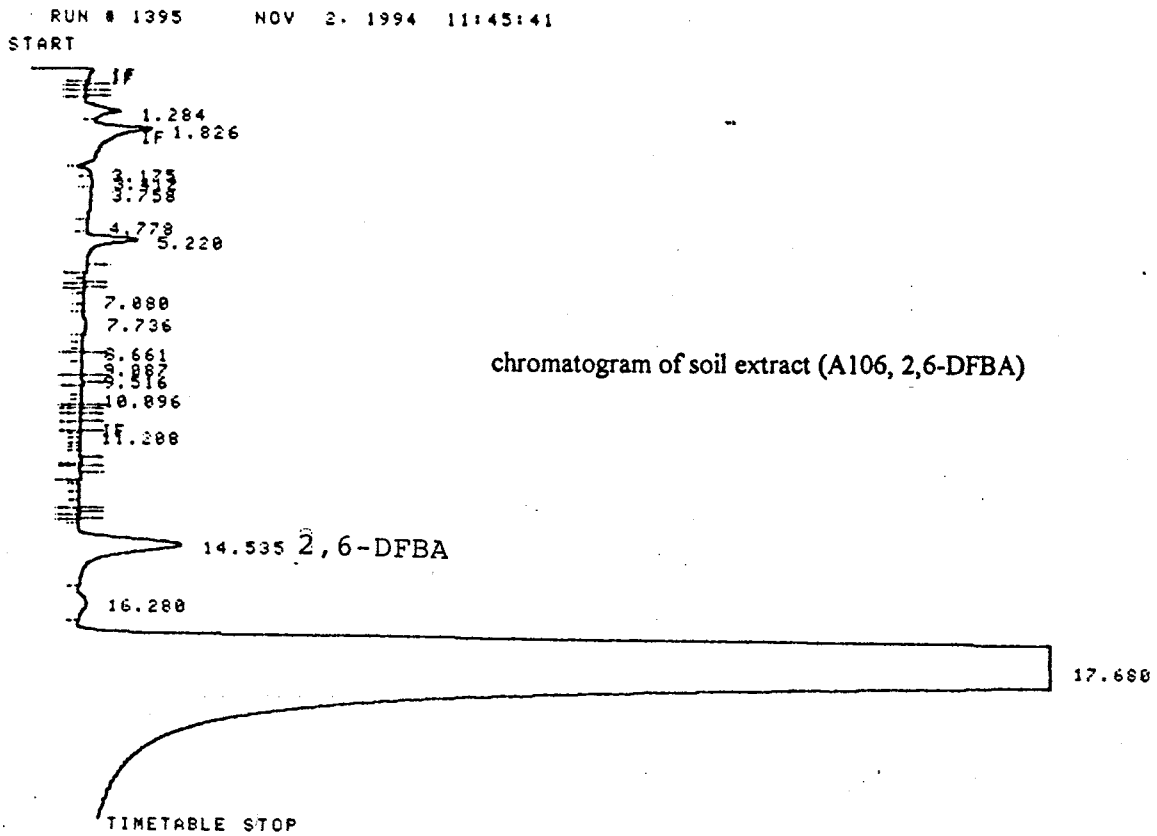
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/HEIGHT
1R	15.598	1	5.0000E+00	2.2309E-04

CAL#	NAME
1	PFBA

CALIBRATION OPTIONS



chromatogram of soil extract (A106, 2,6-DFBA)

RUN# 1395 NOV 2, 1994 11:45:41

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
14.535	1620134	PB	1R	6.756

TOTAL AREA=4.1336E+08
MUL FACTOR=1.0000E+00

CALIBRATION

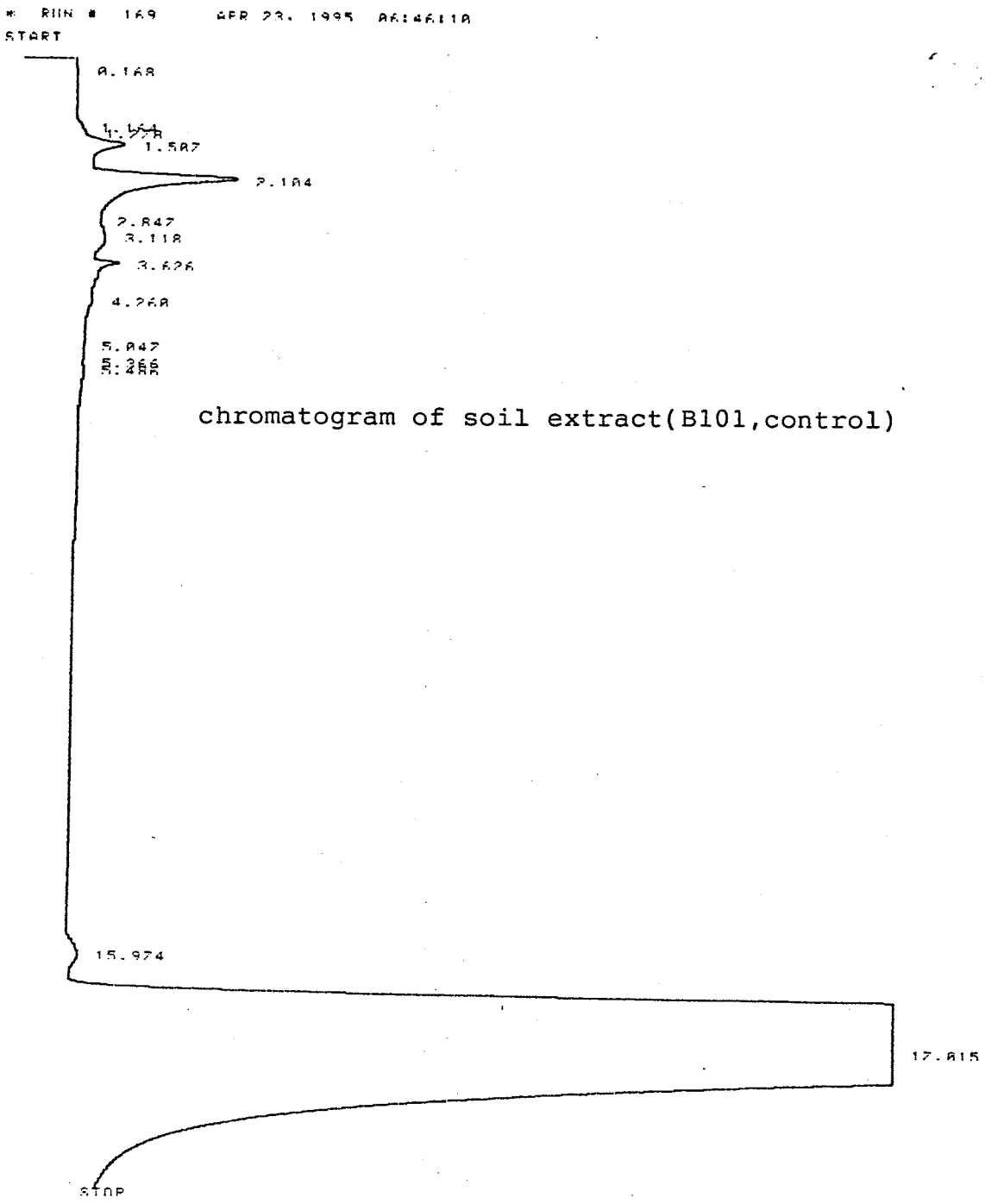
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	14.422	1	5.0000E+00	4.1701E-06

CAL#	NAME
1	2,6-DFBA

CALIBRATION OPTIONS



chromatogram of soil extract(B101,control)

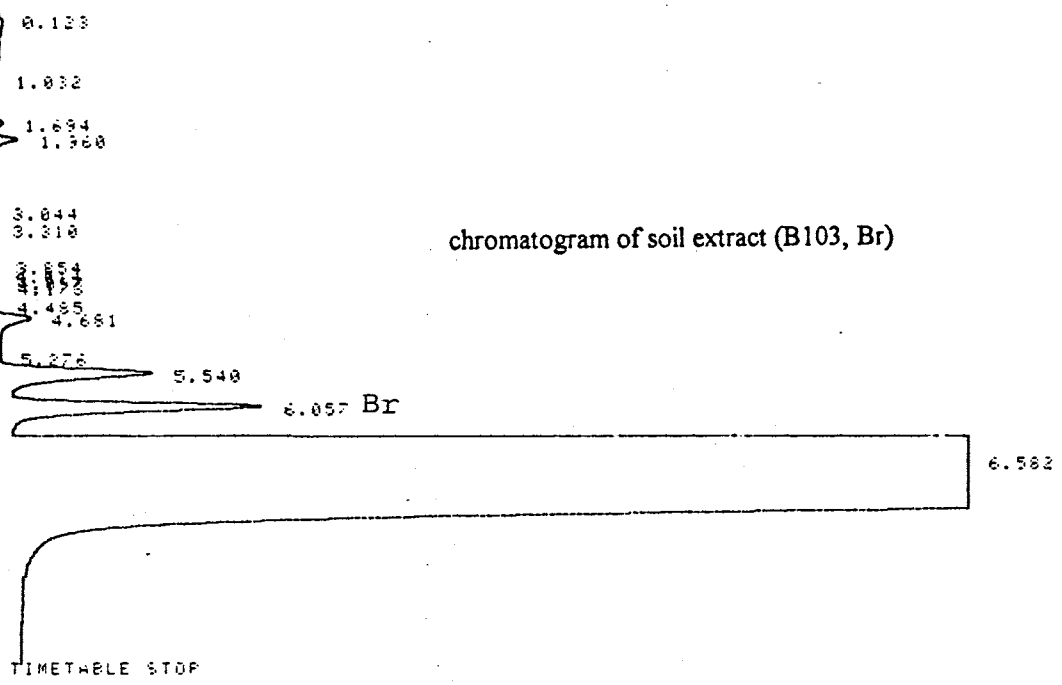
RUN# 169 APR 23, 1995 08:46:10

ESTD-AREA

RT	AREA	TYPE	CAL #	AMOUNT
2.847	32588	VV	1R	.897

TOTAL AREA=4.3603E+08
MULT FACTOR=1.0000E+00

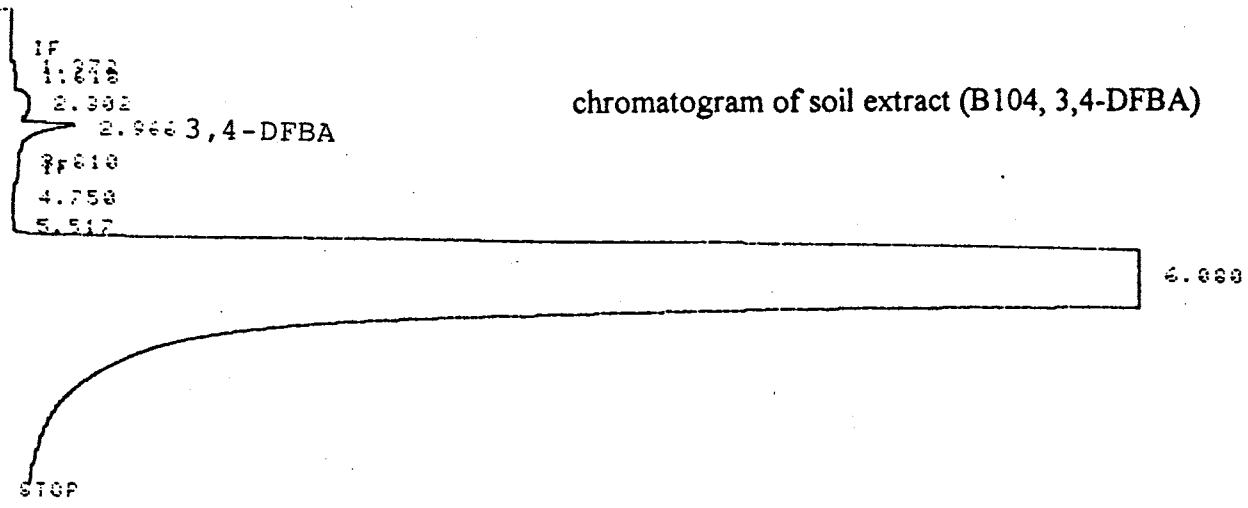
```
*  RUN #    11      JAN 27, 1901   03:05:27
START
```



chromatogram of soil extract (B103, Br)

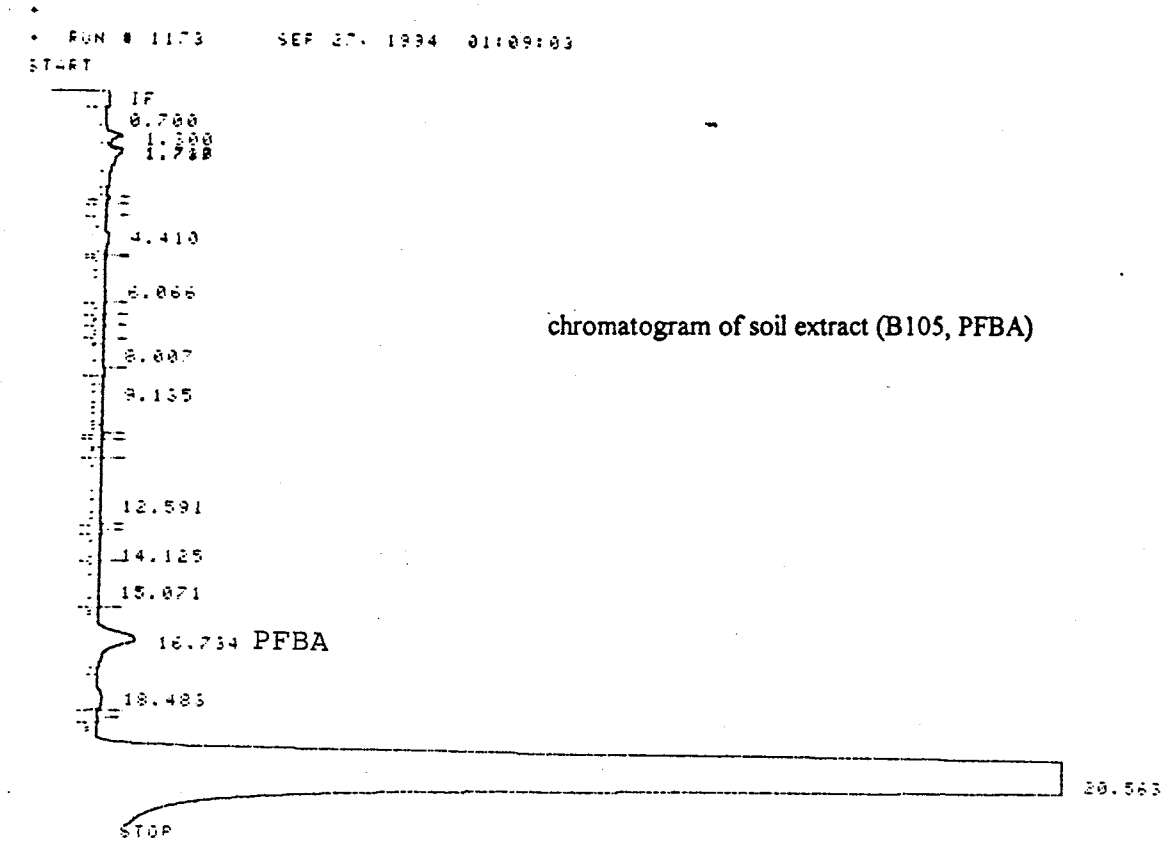
```
RUN#     11            JAN 27, 1901   03:05:27

ESTD-AREA
      RT        AREA TYPE   CAL#        AMOUNT
   6.057     1358459   VB     1R         4.680

TOTAL AREA=3.1854E+08
MUL FACTOR=1.0000E+00




RUN PARAMETERS
ZERO    =  0
ATT 2^  =  6
CHT SP  =  1.0
AR REJ  =  0
THRSH   =  0
PK WD   =  0.04
```

```
*  RUN #  941      AUG 15, 1994  22:21:15
START
```



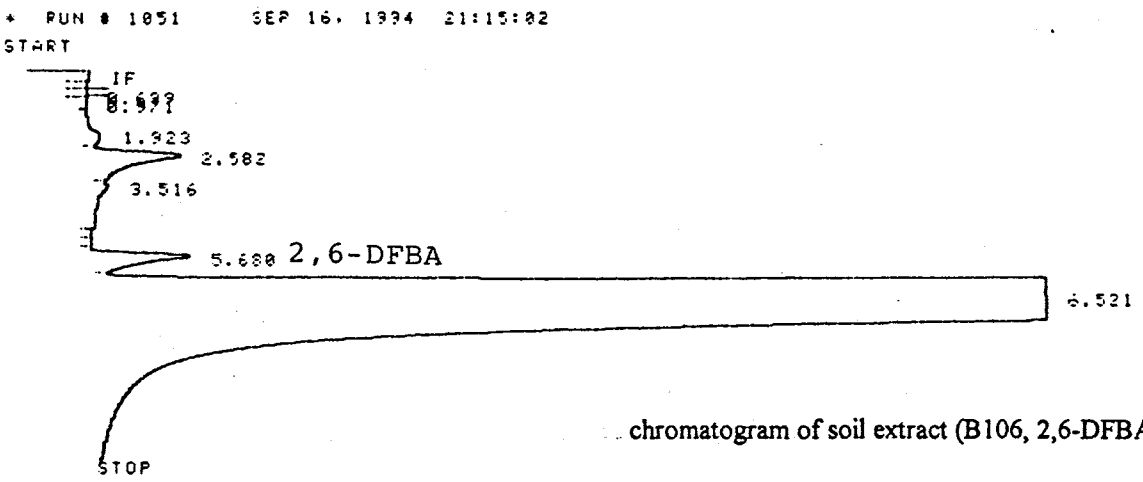
chromatogram of soil extract (B104, 3,4-DFBA)

```
RUN#   941           AUG 15, 1994  22:21:15

ESTD-AREA
      RT        AREA TYPE  CAL#       AMOUNT
   2.966      646960   HH    1R         2.091

TOTAL AREA=3.9811E+00
MUL FACTOR=1.0000E+00



CALIBRATION

ESTD
REF % RTW:   5.000  NON-REF % RTW:   5.000

LEVEL: 1             RECALIBRATIONS: 1

CAL#      RT      LV     AMT       AMT/AREA
  1R    2.983     1 5.0000E+00  4.4681E-06

CAL#      NAME
  1  3,4-DFBA

CALIBRATION OPTIONS
```



chromatogram of soil extract (B105, PFBA)

```
RUN#  1173            SEP 27, 1994  01:05:03

ESTD-AREA
      RT        AREA TYPE  CHL#       AMOUNT
  16.734      579696   VV    1R         3.715

TOTAL AREA=3.2064E+03
AVG RFACTOR=1.0000E+00



CALIBRATION

ESTD
REF % RTW:    5.000  NON-REF % RTW:    5.000

LEVEL: 1              RECALIBRATIONS: 1

CHL#      RT      LV     AMT       AMT AREA
  1R   16.843     1 5.0000E+00  6.4013E-06

CHL#     NAME
  1  PFBA
```



chromatogram of soil extract (B106, 2,6-DFBA)

RUN# 1051 SEP 16, 1994 21:15:02

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
5.680	1273516	VH	1R	4.315

TOTAL AREA=4.0447E+05
MUL FACTOR=1.0000E+00

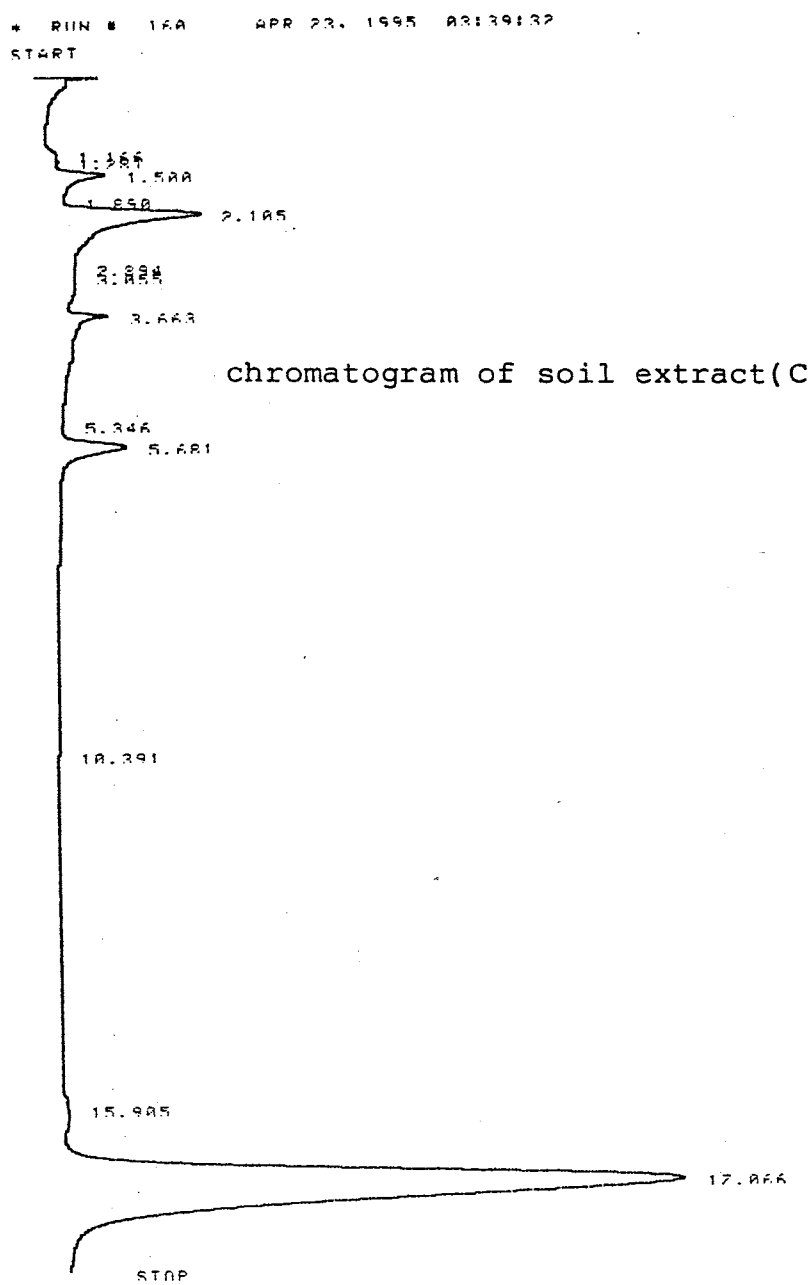
CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	5.718	1	5.0000E+00	3.3861E-06

CAL#	NAME
1	2,6-DFBA



chromatogram of soil extract(C101,control)

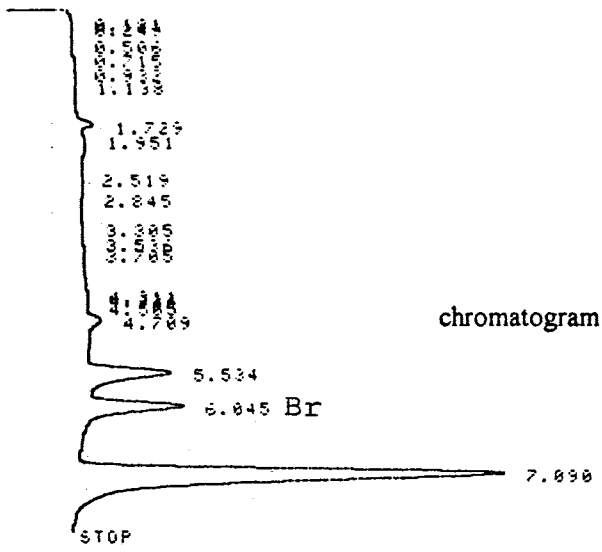
RUN# 1AA APR 23, 1995 03:39:32

ESTD-AREA

RT	AREA	TYPE	CAL #	AMOUNT
2.894	8198	VV	1R	.024

TOTAL AREA=6358000
MUL FACTOR=1.0000E+00

* RUN # 19 JAN 27, 1901 04:28:12 -
START



chromatogram of soil extract (C103, Br)

RUN# 19 JAN 27, 1901 04:28:12

ESTD-AREA

RT	AREA	TYPE	CHL#	AMOUNT
6.045	659527	VB	1R	2.273

TOTAL AREA=4337501
MUL FACTOR=1.0000E+00

RUN PARAMETERS
ZERO = 0
ATT 2^ = 6
CHT SP = 1.0
AR REJ = 0
THRSH = 0
PK WD = 0.04

CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

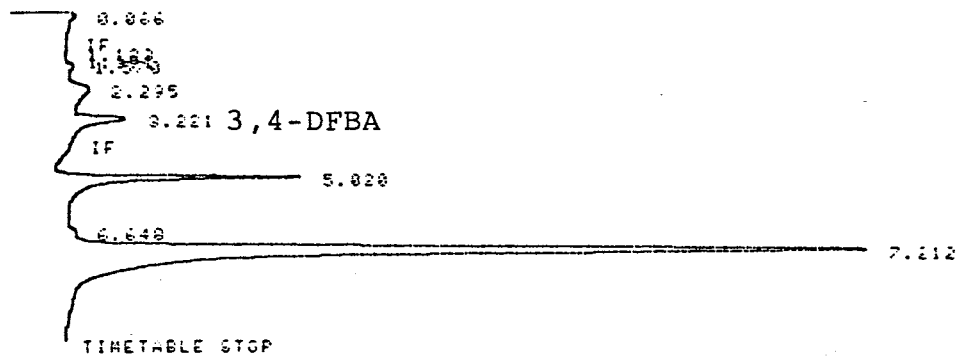
LEVEL: 1 RECALIBRATIONS: 1

CHL#	RT	LV	AMT	AMT/AREA
1R	6.053	1	5.0000E+00	3.4454E-06

CHL#	NAME
1	BR

MUL FACTOR ..... 1.0000E+00

* RUN # 784 JUL 25, 1994 15:46:03
START



chromatogram of soil extract (C104, 3,4-DFBA)

RUN# 784 JUL 25, 1994 15:46:03

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
3.221	719167	HP	1R	2.496

TOTAL AREA=1.1000E+07
MUL FACTOR=1.0000E+00

CALIBRATION

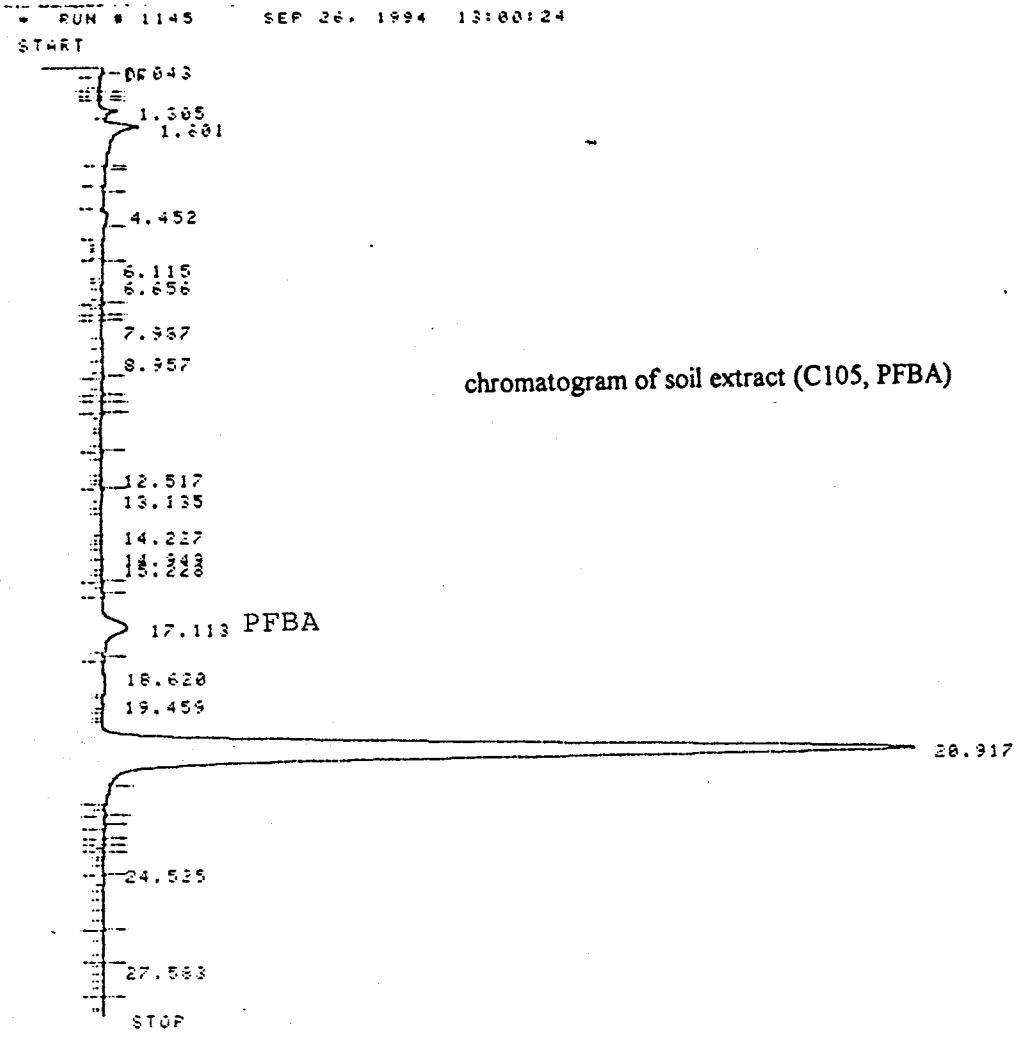
ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
1R	3.224	1	5.0000E+00	3.4700E-06

CAL#	NAME
1	3,4-DFBA

CALIBRATION OPTIONS
RF of uncalibrated peaks .... 0.0000E+00
Calibration fit ..... P
Disable post-run RT update .. NO
SAMPLE AMT ..... 0.0000E+00
MUL FACTOR ..... 1.0000E+00



chromatogram of soil extract (C105, PFBA)

RUN# 1145 SEP 26, 1994 13:00:24

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
17.113	394955	BV	IR	2.586

TOTAL AREA=1.3972E+07
MUL FACTOR=1.0000E+00

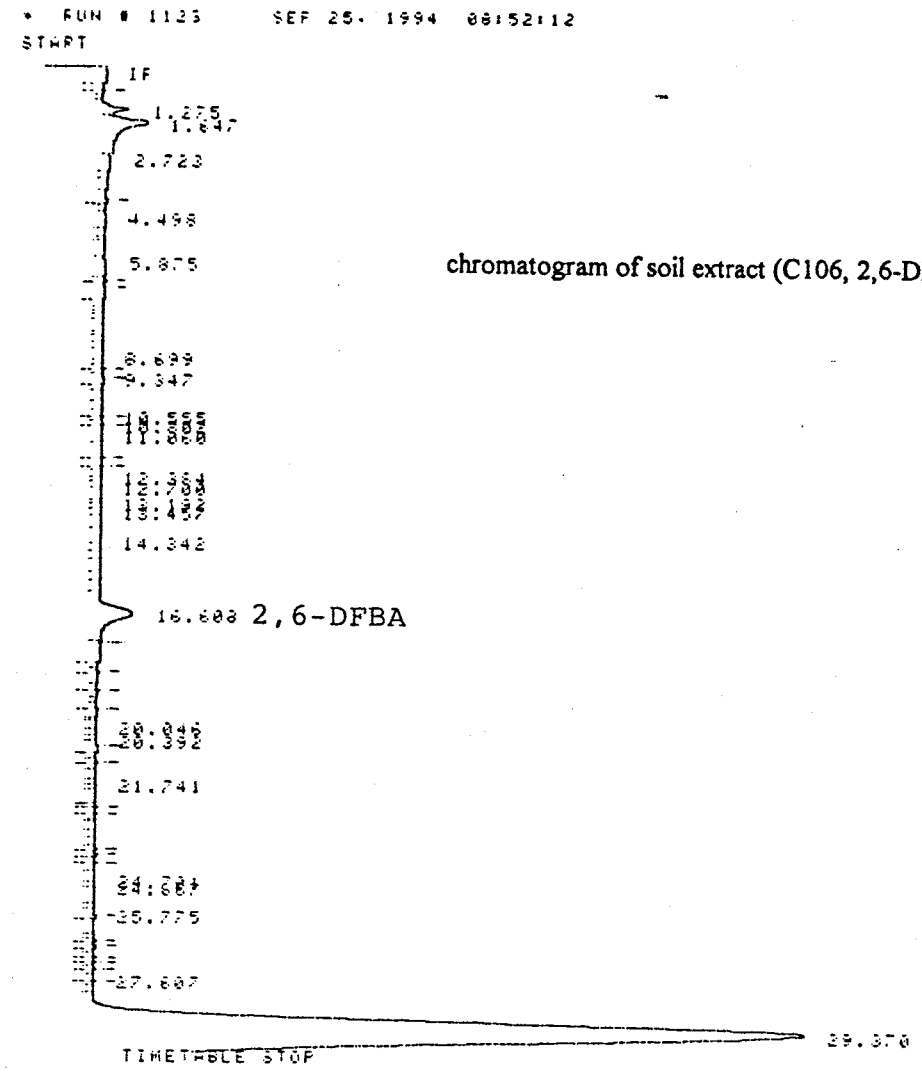
CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	LV	AMT	AMT/AREA
IR	17.092	1	5.0000E+00	6.5472E-06

CAL#	NAME
1	FFBA



chromatogram of soil extract (C106, 2,6-DFBA)

RUN# 1123 SEP 25, 1994 08:52:12

ESTD-AREA

RT	AREA	TYPE	CAL#	AMOUNT
16.608	467600	VP	IR	2.381

TOTAL AREA=1.7303E+07
MUL FACTOR=1.0000E+00

CALIBRATION

ESTD
REF % RTW: 5.000 NON-REF % RTW: 5.000

LEVEL: 1 RECALIBRATIONS: 1

CAL#	RT	Lv	AMT	AMT AREA
IR	16.366	1	5.0000E+00	4.8235E-06