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CHEMICAL DERIVATIZATION ANALYSIS OF PESTICIDE RESIDUES. XI. AN IMPROVED METHOD FOR THE DETERMINATION AND CONFIRMATION OF ACIDIC HERBICIDES IN WATER

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Chemical Derivatization Analysis of Pesticide Residues. XI. An Improved Method for the Determination and Confirmation of Acidic Herbicides in Water

by

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MANAGEMENT PERSPECTIVE

In response to requests made by Water Quality Branch, Western and Northern Region, and the National Water Quality Laboratory, an improved method was developed for the determination of 13 acidic herbicides in water. The major source of interference in the existing procedure was identified and a cleanup step was included to alleviate the problem. The new method significantly decreases the number of false identifications and thereby reduces the workload of the mass spectrometer for confirmation purposes. It also increases the cost effectiveness by extending the applicability of the method to more herbicides.

Dr. J. Lawrence
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PERSPECTIVE-GESTION

En réponse à une demande formulée par la Direction de la qualité des eaux, la Région de l'ouest et du nord et le Laboratoire national de la qualité des eaux, nous avons mis au point une nouvelle méthode améliorée permettant de doser 13 herbicides acides dans l'eau. Nous avons identifié la principale source de perturbations dans la méthode existante et nous avons prévu une étape de purification destinée à diminuer l'importance de ce problème. Cette nouvelle méthode permet de diminuer sensiblement le nombre de fausses identifications et ainsi de réduire l'utilisation du spectromètre de masse à des fins de confirmation. Elle permet aussi d'améliorer la rentabilité, car la méthode peut alors s'appliquer à un plus grand nombre d'herbicides.

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ABSTRACT

A procedure for the determination and confirmation of acidic herbicides in water is described. This method is applicable to 13 commonly used herbicides, including the monochlorinated ones, at μg/L and sub-μg/L levels. The water sample is acidified to pH<2 and extracted by dichloromethane. The herbicides are converted into their pentafluorobenzyl (PFB) derivatives and the products are cleaned up on a silica gel column. A gel permeation cleanup using a Bio-Beads S-X3 column is included to remove the PFB esters of fatty acids which are the major interferences in the final analysis using electron capture detection. Confirmation of herbicides employing electron impact and negative ion chemical ionization mass spectrometry is discussed and applied to river water samples. The mean recovery of herbicides for water samples fortified at 1 and 0.1 μg/L ranges from 45 to over 90%. The detection limit of herbicides in natural water samples is 0.05 μg/L with electron capture detection and 0.02 μg/L with negative ion chemical ionization mass spectrometry.

RÉSUMÉ

On décrit une méthode permettant de doser et de confirmer la présence des herbicides acides dans l'eau. Cette méthode s'applique à 13 herbicides couramment utilisés, y compris les herbicides monochlorés, à des concentrations de l'ordre du µg/L et à des concentrations plus faibles. On acidifie l'échantillon d'eau jusqu'à un pH inférieur à 2, puis on extrait avec du dichlorométhane. On obtient les dérivés pentafluorobenzyliques (PFB) des herbicides, puis on purifie les produits sur une colonne de gel de silice. On prévoit une étape de purification par perméation sur gel à l'aide d'une colonne de Bio-Beads S-X3, afin d'éliminer les esters pentafluorobenzyliques des acides gras; ce sont ces esters qui constituent les perturbations principales au cours du dosage final par détection par capture d'électrons. On examine la confirmation de la présence des herbicides par spectrométrie de masse à impact électronique et à ionisation chimique à ions négatifs et on l'applique à l'analyse d'échantillons d'eau de rivière. La valeur moyenne du taux de récupération des herbicides dans des échantillons d'eau fortifiés à 1 µg/L et à 0,1 µg/L varie de 45 % à plus de 90 %. La limite de détection des herbicides dans des échantillons d'eau naturelle est de 0,05 µg/L, lorsque le dosage est réalisé par détection par capture d'électrons, et de 0,02 µg/L lorsque l'analyse est effectuée par spectrométrie de masse à ionisation chimique à ions négatifs.

INTRODUCTION

Phenoxyalkanoic and related herbicides are used for the control of terrestrial and aquatic weeds in many agricultural and urban applications [1,2]. Because of their persistence, these toxic herbicides remain in the environment and could cause hazards to human health or jeopardize the survival of fish and wildlife. As a result, a great deal of effort has been devoted to the development of analytical methods for these herbicides in water and other matrices and the methodologies are regularly reviewed [3]. In many cases, gas chromatographic determination with an electron capture detector (ECD) was performed after the acids are converted into esters using one of the following approaches: (a) formation of methyl [4,5] or butyl [6] esters by reaction with diazomethane or other suitable alkylating agents, (b) formation of alkyl esters containing fluorine or chlorine atoms such as the 2,2,2-trifluoroethyl (TFE) [7], 2,2,3,3,3-pentafluoropropyl (PFP) [8], 2-chloroethyl (CE) [9], and 2,2,2-trichloroethyl (TCE) [10] derivatives with the corresponding alcohols, and (c) formation of pentafluorobenzyl (PFB) esters [11-14] by reaction with α-bromo-2,3,4,5,6-pentafluorotoluene (pentafluorobenzyl bromide, PFBBr).

Although each method has its own applications, the methyl ester procedure using the diazomethane reagent is the most popular one since the reaction is quantitative for nearly all acidic herbicides and the products are stable and relatively free from interference in the final ECD analysis. However, this procedure lacks the sensitivity for all monochlorinated herbicides and it is not applicable to environmental samples contaminated by those compound at low μg/L levels. Previously, we have developed methods for the determination of acidic herbicides [15], phenols [16], resin and fatty acids [17] in water or effluent samples at μg/L levels by the formation of their PFB derivatives. The

latter are among the most sensitive derivatives for EC detection and thus more suitable for the determination of non-chlorinated and mono-chlorinated pollutants in the environment. Based on the PFB derivatives, we present an expanded and improved multi-residue procedure that can replace our previous method for acidic herbicides, with refinements on sample cleanup, confirmation of compound identity and quality assurance. The 13 herbicides examined in this method are listed in Table 1.

EXPERIMENTAL

Reagents

(a) Solvents.--Use only distilled-in-glass, pesticide residue grade solvents and check before use for low blank values.

(b) Organic-free water.--Pass distilled water through a 4-cartridge Millipore Super Q water purification system.

(c) Standards and solutions.--PFBBr, 2,3,4,5,6-pentafluorophenoxyacetic acid, 2,3-dichlorophenoxyacetic acid (2,3-D) and 3,5-dibromo-4-hydroxybenzonitrile (bromoxynil) are products of Aldrich Chemicals Co. (Milwaukee, WI). Other herbicides are either obtained from USEPA or from their manufacturers.

Prepare 1000 μg/mL stock solution of each herbicide by dissolving 100 mg of the analytical standard in 100 mL of acetone in a low actinic volumetric flask. For fortification purpose, prepare mixed herbicide stock solutions of 10 and 1 μg/mL in acetone by combining appropriate aliquots of individual stock solutions and diluting to 100 mL. Keep all solutions in crimp-top vials at -20°C in the dark. Prepare a 5% PFBBr solution by dissolving 1 g of the chemical in 20 mL of acetone with a water content of 0.2% (v/v) or less. Keep the reagent

at -20°C in the dark.

(d) Sodium sulfate.--Anhydrous, reagent grade (Fisher Scientific). Heat at 600°C for 16 hr and store in sealed glass bottles.

(e) Silica gel.--GC grade 950, 60-200 mesh (Fisher Scientific). Activate by heating at 130°C for 16 hr then deactivate by adding 5 mL of organic-free water to 95 g of the adsorbent. Mix well by tumbling and equilibrate overnight in a tightly capped glass container before use. Prepare fresh biweekly.

(f) K_2CO_3 solution.--30% in organic-free water.

Sampling

Collect grab samples of river water in 1 L glass bottles with Teflon liners. Adjust pH of water to 2 or less (pH paper) with 1:1 H_2SO_4. Keep samples at 4°C in the dark and extract them as early as possible.

Extraction and derivatization

Mark the meniscus on the bottle and measure the volume of the water sample to the nearest 5 mL at the end of the extraction by refilling the bottle to the mark and transferring to a 1000 mL graduated cylinder. Check the pH of water to ensure that it is less than 2. Spike the sample with 100 μL of a 10 μg/mL solution of 2,3-D in acetone. Using a magnetic stirrer, extract the sample vigorously with 50 mL of dichloromethane (DCM) for 30 min. Transfer the sample to a 1 L separatory funnel and separate layers. If an emulsion forms, leave the emulsion with the aqueous layer. Repeat the extraction twice and drain the emulsion into the organic layer after the last extraction. Decant the combined organic extract to another flask so that water in the organic layer is left behind in the original flask. Rinse the original flask twice with 5 mL aliquots

of DCM and add the rinsings to the combined extract. Evaporate the solvent to near dryness with a rotary evaporator and a water bath at 40°C. Transfer the residue to a test tube with four 1 mL acetone rinses. Reduce the volume of acetone to 1 mL in a 50°C bath using a gentle stream of nitrogen. Add 100 μL of the 5% PFBBr reagent and 30 μL 30% K_2CO_3 and mix well. Tightly stopper the tube and heat the mixture at 60°C for 1 hr. At the end of the reaction, evaporate the acetone to dryness with nitrogen and redissolve residue in 1 mL of hexane.

Silica gel column cleanup

Fill a 1.0 cm I.D. x 50 cm chromatographic column with 5.00 g of 5% deactivated silica gel and 1 cm of anhydrous sodium sulfate at the top. Elute the column with 20 mL hexane and discard the washing. Transfer the derivatized extract to the column with 3 x 1 mL hexane rinsings. Elute the column with 50 mL 10% DCM in hexane and discard this fraction. Continue the elution with 75 mL DCM and collect this fraction. Concentrate this fraction to ca. 5 mL with a rotary evaporator and then to 0.5 mL with a gentle stream of nitrogen.

Gel permeation chromatographic (GPC) cleanup

Soak 60 g of Bio-Beads S-X3 (200-400 mesh, Bio-Rad) for 24 hr. in 100 mL of the GPC solvent, a 55/45 (v/v) DCM and hexane mixture. Pack the swollen gel into a 2.5 cm I.D. x 50 cm glass column with Teflon end fittings and plungers (Analytical Bio-Chemistry Laboratories, Columbia, Missouri). Install the top plunger and compress the packing slightly to ca. 40 cm length by forcing excess solvent out through the bottom plunger. Connect the column inlet to a loop injector (Waters Associates, Model U6K) with a 1 mL loop which is attached to a HPLC pump (Waters Associates, Model 510) and the outlet to a fraction collector

(Gilson, Model 201). Adjust the flow rate to 2.5 mL/min. Inject the sample after silica gel column cleanup onto the GPC column. Discard the first 115 mL and collect the next 85 mL fraction. Add 1 mL of iso-octane, evaporate the second fraction in two steps to ca. 0.5 mL as described above and readjust the final volume to 1.0 mL with iso-octane.

Instrumentation

For GC-ECD and electron impact mass spectral (EI-MS) work, a Hewlett-Packard 5880A GC equipped with a split/splitless injector, a Ni-63 electron capture detector and a model 5970B Mass Selective Detector (MSD) was used. For negative ion chemical ionization mass spectral (NICI-MS) work, a Finnigan INCOS 50 system was used. See Table 2 for chromatographic conditions.

Acquisition of Mass Spectral Data

Obtain full scan EI-MS data by scanning the MSD from m/z 50 to 470 at a rate of 1.0 scan/s and a scan threshold of 1000. Electron energy and electron multiplier voltage are 70 eV and 2000 V, respectively. Use the quantitation and confirmation ions of the herbicide PFB esters (see Results and Discussion) to acquire data in the selected ion monitoring mode. For NICI-MS work, acquire limited full scan data by scanning from m/z 180 to 280 using hydrogen (0.15 torr) as a reagent gas.

Calibration standards and calculations

For standards, derivatize known amounts of herbicides at two or more concentration levels, make up in iso-octane and omit the cleanup steps. Also derivatize 50 μg of pentafluorophenoxyacetic acid, a compound which is not found

in the environment, and make up to 5.0 mL in iso-octane. Spike 50 μL of this 10 ng/μL internal standard solution to each calibration standard and sample extract just before GC-MS determination.

Use external standard calibration procedure for all ECD work and calculate the concentration of a parameter in the sample from a calibration curve of at least two points. For GC-MS work, follow the internal standard calibration protocol as detailed in USEPA Method 625 [18]. Determine the response factor (RF) of each herbicide PFB ester against the PFB ester of pentafluorophenoxy-acetic acid and use the RF to calculate the concentration of each parameter in the sample.

RESULTS AND DISCUSSION

Selection of a derivative for herbicides analysis

In order to meet the objectives for the surveillance and monitoring of herbicides in receiving waters, it is necessary to develop a routine GC-ECD method with a low detection limit for all herbicides. Particularly, the method must have good sensitivity for the monochlorinated compounds. In the process of selecting a derivative for herbicide analysis, we have prepared the PFB, PFP, TCE and methyl derivatives of the 13 herbicides and their relative response factors (RRF, ECD response of herbicide derivative relative to the response of PFB ester of pentafluorophenoxyacetic acid on equal weight basis) were calculated. For conciseness, the RRF of three representative herbicides containing, respectively, one, two and three chlorine atoms, i.e.: MCPA, 2,4-D, and 2,4,5-T, are tabulated in Table 3. Also included for comparison is the RRF of palmitic acid, a non-chlorinated fatty acid. Note that the TFE and CE derivatives were not evaluated

because, with a smaller number of halogen atoms, the ECD would be less sensitive to them as compared to the PFP and TCE derivatives, respectively. The butyl esters were also omitted since they were less commonly used and their RRF would be similar to the corresponding methyl esters. A quick glance of Table 3 indicated that the methyl esters had, as expected, the lowest RRF's of all derivatives. In particular, the methyl ester of MCPA was not detected by the ECD even at high pg levels. For this reason, this derivative is not applicable to the determination of MCPA and other monochlorinated herbicides at residue levels. Somewhat surprisingly, the RRF for the PFP ester of MCPA was also very low despite the fluorine and chlorine atoms on the ester and thus this derivative is also unsuitable for the monochlorinated herbicides. In contrast, high ECD sensitivity was observed for the PFB and TCE derivatives of all herbicides and the RRF's were within a factor of two regardless of the number of chlorine atoms in the original acids. However, the RRF's for the PFB esters were about twice as large as those of the corresponding TCE esters. Also, trichloroethanol did not react with bromoxynil and the yield of the 2,3,6-TBA derivative was poor, thus the PFB esters were the only derivatives best suited for the simultaneous determination of all 13 herbicides at residue levels.

Extraction, derivatization, silica gel cleanup and gas chromatography

In comparison to our previous version [15], the following changes have been incorporated into the present method. The procedure was extended to include 4-CPA, MCPP and bromoxynil. Bromoxynil is a phenol and it was converted into its PFB ether derivative and determined alongside the other acidic herbicides. Attempts had also been made to include trichloroacetic acid and dinoseb into the same procedure but they were unsuccessful because the PFBBr

reagent did not react with these two herbicides.

To monitor the recovery of herbicides in environmental samples processed by this procedure, a known amount of 2,3-D was added as a surrogate to each water sample prior to extraction. This compound was chosen since it has similar analytical properties to many phenoxyalkanoic herbicides and also it is not present in the environment. The volume of acetone used in the derivatization step was reduced to 1 mL from 4 mL in our previous procedure. This smaller volume gave more reproducible yields of the herbicide derivatives at sub-microgram levels. In order to have a more reproducible elution pattern, a 5 g deactivated silica gel column was used in place of a miniature column. The change enabled us to standardize the cleanup procedures for the PFB derivatives of phenols, resin and fatty acids as well as the acidic herbicides. With the present procedure, all herbicide derivatives were eluted in one fraction instead of two in the past and thus instrumental analysis time was reduced to a half.

As shown in Figure 1, the PFB derivatives of the 13 herbicides and the surrogate were adequately resolved by a 30 m DB-5 capillary column using the described temperature program. Two peaks were observed for our sample of 2,3,6-TBA and their mass spectra were nearly identical, indicating that the minor component is also an isomer of TBA. However, the lack of authentic standards precluded us from elucidating its exact isomeric form. If GC analysis on a second column is required, then a DB-1 column is recommended. The herbicide derivatives and coextractives had a different elution pattern on this non-polar column and therefore it was useful for tentative compound identification. In contrast, the DB-17 column was less useful for herbicide analysis as it was unable to separate the esters of MCPA and 2,4-DP as well as those of MCPB and 2,4,5-T.

Interferences

A major disadvantage of the PFB ester procedure is the large amount of interfering substances present when the extract is analyzed by an ECD. In many cases, the final analysis has to be performed on two or three capillary columns of different polarity to separate the coeluting interference peaks. Attempts to remove those interferences from the herbicides by silica gel column cleanup were unsuccessful. A closer look at those peaks by EI and EC-NICI-MS revealed that most of the major interference peaks were saturated and unsaturated fatty acids. Fatty acids are coextracted with the herbicides and are also converted into their PFB esters in the derivatization step. Since the RRFs of the PFB esters of palmitic acid (Table 1) as well as other fatty acids are similar to those of the herbicides, presence of the fatty acids at μg/L levels or higher would pose an interference problem. Fatty acids are ubiquitous in the environment and a large number of them from C_1 to C_{24} in saturated, unsaturated, straight chain and branched chain forms have been reported in natural water and rain samples [19,20]. Among these fatty acids, those from C_{12} to C_{18} are in the same retention time window as the herbicides and they will interfere when PFB esters are formed. Unfortunately, these acids, particularly those with even carbon numbers, are also the predominant ones occurring at concentrations ten to a few hundred times higher than the herbicides in environmental samples.

The other source of fatty acids is of laboratory origin. A major one arises from the anhydrous sodium sulfate used for the removal of water in the organic extract. We have tested batches of the adsorbent from three different suppliers and all of them were contaminated with high ng/g amounts of various fatty acids, including lauric, myristic, palmitic, palmitoleic, stearic, oleic and linoleic, to name just a few. The number and amounts of fatty acids present

were enough to severely interfere with the determination of herbicides in water samples. Heating the adsorbent at 600°C for 16 hr could only remove 60 to 70 % of the fatty acids. For this reason, the use of sodium sulfate before the derivatization step in this procedure should be avoided wherever possible.

Gel permeation cleanup of extracts

Gel permeation chromatography (GPC) has long been used for the separation of lipids and fatty acids from other xenobiotic compounds in fish and fatty samples [21]. GPC was also effective for the removal of fatty acid interferences in herbicide extracts. In this procedure, the cleanup was performed after the derivatization step so that all fatty acids native in the water sample as well as those introduced from the laboratory could be removed. Also, the GPC step could be optional and it was needed only if the ECD trace showed excessive interference. Using a 60 g Bio-Beads S-X3 column and the conditions as described in the Experimental section, PFB esters of fatty acids with 12 carbons or more are completely separated from the herbicides. However, fatty acids with a shorter carbon chain and benzoic acids were not separated from the herbicides in their PFB ester form and thus could still interfere.

GC-MS analysis and confirmation of herbicides

The other approach to solve this interference problem is to use more selective detection techniques such as EI and EC-NICI mass spectrometry. Under standard EI conditions, the base peak or the second most intense peak in the mass spectrum of each herbicide derivative was always the PFB ion, $(C_6F_5CH_2)^+$, of m/z 181 and this is consistent with the results reported previously by de Beer [22]. The characteristic ions and their relative intensities of these derivatives are

tabulated in Table 4. Because of the differences in molecular structure, the fragmentation patterns of these herbicide derivatives are also different. For the substituted phenoxyacetic and phenoxypropionic acids, the molecular ions and the $(M\text{-}PFB\text{-}CO_2)^+$ ions were of moderate to strong intensities. The derivative of each phenoxyacetic acid further fragmented to give the aromatic moiety (Ar^+) which was characteristic of the parent herbicide. The derivative of each phenoxypropionic acid, however, produced the phenolic ion ($ArOH^+$) which was again characteristic of the parent compound. In contrast, the molecular ions of the two phenoxybutanoic acid derivatives were very weak and the non-characteristic $(C_6F_5CH_2CO_2C_3H_6)^+$ ion was produced in both cases. The two benzoic acid derivatives exhibited molecular ions of moderate intensities and benzoyl ions ($ArCO^+$) of moderate to strong intensities. For dicamba, the $ArCO^+$ ion further eliminated CH_2 from the methoxy group to give the $C_6H_2Cl_2(OH)CO^+$ ion of m/z 189. For TBA, the $(M\text{-}Cl)^+$ ion of m/z 369 was also observed. The molecular ions of the PFB derivatives of picloram and bromoxynil were also very weak. While the picloram derivative exhibited major fragments of m/z 224 and 196, attributable to the loss of NH and then CO from the $(M\text{-}PFB)^+$ moiety, no significant characteristic ion was observed for the PFB ether of bromoxynil. Thus, with the exception of MCPB, 2,4-DB and bromoxynil, confirmation of the herbicides in water samples can be accomplished by EI-MS in selected ion monitoring mode using the characteristic ions listed in Table 4, provided that their levels are above 0.1 μg/L (assuming a concentration factor of 1000).

For the quantitation and confirmation of all herbicides at lower levels, a more sensitive technique is required. Recently, electron capture NICI-MS has been successfully applied to the analyses of the PFB derivatives of chlorophenols [23,24], chloroanilines [24] and resin acids [17] as well as some

fluorinated derivatives of pesticides [7]. When this soft ionization technique was applied to the herbicide esters, a simple mass spectrum consisting of strong ions characteristic of the $(M-181)^-$ cluster was observed for each ester (Table 5). The molecular ion, M^-, was either absent or very weak (less than 10% relative abundance). If a limited full scan mode from m/z 180 to m/z 280 is used, all herbicides can be quantified and confirmed with a sensitivity similar to an ECD. Therefore, EC-NICI-MS is the most efficient technique for the determination of the PFB derivatives and it is being used in our water quality laboratories for the confirmation of acidic herbicides in water extracts.

Method performance in fortified water samples

To evaluate the performance of this procedure, one litre subsamples of a river water with undetectable herbicides blanks were fortified to 1 and 0.1 μg/L levels with the 13 herbicides and replicate analyses were performed. Using ECD for final analysis, the precision and accuracy of this method including GPC cleanup are summarized in Table 6. The overall recoveries for various herbicides ranged from ca. 45% to over 90% and they were generally related to their dissociation constants and solubilities in water (Table 1). For those herbicides with lower solubilities and/or higher pK_a's such as 2,4-DP, silvex, bromoxynil, MCPB, and 2,4-DB, the recoveries were up to about 90%. In contrast, recoveries from 50 to 70% were obtained for herbicides with higher solubilities and/or lower pK_a's such as 4-CPA, dicamba and 2,3,6-TBA. The recovery of picloram was very low (45 to 50%) despite its moderate solubility. A plausible explanation is that the amino group of this herbicide is protonated into a more soluble anilinium ion under the acidic extraction conditions of pH 2 or less. Losses in the GPC step were ca. 5 to 10 % and were mostly due to incomplete transfer of the sample prior

to injection. The relative standard deviations for replicate analysis were between 3% and 10% at 1 and 0.1 μg/L. The mean recovery of the surrogate, 2,3-D, was 71% (Table 6). For an on-going in-house quality assurance program, a laboratory must determine its own surrogate recovery and set an acceptable limit for it in the samples. If the recovery of the surrogate is outside of this acceptable range, the analytical problem must be identified and corrected.

Application to environmental samples

The newly developed procedure has been applied to the determination of acidic herbicides in water samples collected from three locations in Manitoba. These samples were analyzed before and after GPC cleanup. Based on the retention time obtained on a single capillary column, 2,4-D was tentatively identified in all samples before GPC cleanup. Upon repeated analysis after GPC cleanup, 2,4-D was present in only three of the samples. The artifact was later identified as tridecylic acid by GC-MS using an authentic standard. We have also observed from other samples that, under some GC conditions, the PFB esters of lauric acid and MCPA as well as those for myristic acid and silvex, also had very similar retention times that could lead to misidentification. All of the above-mentioned fatty acid artifacts were effectively removed by GPC. Among the samples screened, herbicides were detected in ten incidents by GC-ECD and all of them were subsequently confirmed by GC-MS. The most contaminated sample, 90MAN06, was collected from the Red Deer River at North Perimeter and MCPP, MCPA, dicamba, 2,4-D and bromoxynil were tentatively identified by GC-ECD (Figure 2). The presence of these herbicides except bromoxynil was confirmed by EI-MS using a mass selective detector (Figure 3). With NICI-MS, the presence of all five herbicides in the sample was confirmed. The levels of MCPP, dicamba, MCPA, 2,4-D

and bromoxynil in sample 90MAN06 were, 0.17, 0.12, 1.14, 1.18 and 0.24 μg/L, respectively. The estimated detection limit for the herbicides in water is ca. 0.05 μg/L using ECD and ca. 0.02 μg/L by NICI-MS.

Conclusions

Among the derivatives tested, the PFB esters are most sensitively detected by the ECD and can meet the requirements for the determination of all herbicides including 4-CPA, MCPA, MCPP and MCPB at sub μg/L levels. The majority of interferences in this procedure is attributed to the fatty acids, either naturally occurring in the sample or latter introduced in the laboratory. GPC cleanup significantly minimizes these interferences and thereby reduces the number of false identification in ECD analysis. If a more selective detection technique such as EI-MS or NICI-MS is used instead, the optional GPC step can be omitted. Owing to its high sensitivity and selectivity, NICI-MS is the detector of choice for the determination of these herbicides in water samples at or near the detection limits. The inclusion of a surrogate prior to extraction and an internal standard prior to GC-MS analysis are additional QA measures to improve the quality of data generated by this method.

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LIST OF FIGURES

TABLE 1

Some physical data for the selected acidic herbicides[1,2,25]

Herbicide	FW	mp(°C)	pK_a	Solubility in water (ppm)
4-CPA	186.6	160	2.95	960
MCPP	214.6	92-94	3.75	600-895
Dicamba	221.0	114-116	1.94	4500-7900
MCPA	200.6	118-119	3.05	550-1600
2,4-DP	235.1	118	3.00	180-710
2,3,6-TBA	225.5	125-126	2.6	8400
2,4-D	221.0	140-141	2.73	400-900
Bromoxynil	276.9	189-191	4.06	100-130
2,3-D	221.0	173-175	--	340
Silvex	269.5	179-181	2.84-4.41	140-150
2,4,5-T	255.5	154-155	2.88	200-280
MCPB	228.7	100-101	4.80	44-48
2,4-DB	249.1	120-121	4.80-5.95	46-53
Picloram	241.5	233d	2.95	430

TABLE 2

Chromatographic conditions for the determination of herbicide PFB esters

	ECD/MSD	NICI-MS
Column	J&W DB-5, 30 m x 0.25 mm, 0.25μ thickness	PTE-5, 30 m x 0.25 mm, 0.25μ thickness
Column initial temp., °C	70 for 0.75 min	80 for 5 min
Programming rate 1	30°/min (from 70 to 160)	10°/min (from 80 to 140)
Programming rate 2	2°/min (from 160 to 240)	5°/min (from 140 to 250)
Column final temp., °C	240	250
Detector temp., °C	300 (ECD)	N/A
Ion source temp., °C	200 (MSD)	100
Injection port temp., °C	250	250
Splitless time, min	0.75	0.5
Column head pressure, psi	15 (ECD), 10 (MSD)	12
Injection volume, μL	2	1
Carrier gas	helium	helium
ECD make-up gas and flow	argon/methane (95/5), 25 mL/min	N/A

TABLE 3

Response factors (mean of three trials) for various derivatives of MCPA, 2,4-D, 2,4,5-T and palmitic acid relative to the PFB ester of pentafluorophenoxyacetic acid (=1.000)

Herbicide	PFB	TCE	PFP	Methyl
MCPA	1.175	0.578	<0.002	<0.002
2,4-D	1.320	0.667	0.363	0.113
2,4,5-T	1.333	0.690	0.550	0.481
16:0	0.840	0.380	<0.002	<0.002

TABLE 4

Mass number (m/z) and relative abundance (%) of some characteristic ions observed for the PFB esters of acidic herbicides under EI conditions

Group 1 : Phenoxyacetic acids				
Herbicide	M^+	$[M\text{-}PFB\text{-}CO_2]^+$	Ar^+	PFB^+
4-CPA	366(65) C*	141(100) Q*	111(86)	181(100)
MCPA	380(63) Q	155(50) C	125(83)	181(100)
2,4-D	400(34) C	175(65) Q	145(28)	181(100)
2,4,5-T	434(18) C	209(36) Q	179(14)	181(100)
Group 2 : Phenoxypropionic acids				
Herbicide	M^+	$[M\text{-}PFB\text{-}CO_2]^+$	$ArOH^+$	PFB^+
MCPP	394(63) C	169(100) Q	142(53)	181(92)
2,4-DP	414(26) C	189(66) Q	162(74)	181(100)
Silvex	448(16) C	223(42) Q	196(44)	181(100)
Group 3 : Phenoxybutanoic acids				
Herbicide	M^+	$[PhCH_2CO_2C_3H_6]^+$	PFB^+	
MCPB	408(2)	267(20)	181(100)	
2,4-DB	428(1)	267(23)	181(100)	
Group 4 : Benzoic acids				
Herbicide	M^+	$ArCO^+$	PFB^+	Others
Dicamba	400(28)	203(73) Q	181(100)	189(54) C
2,3,6-TBA	404(13)	207(35) Q	181(100)	369(34) C
Group 5 : Miscellaneous				
Herbicide	M^+	PFB^+	Others	
Picloram	420(1)	181(100)	224(17) C, 196(78) Q	
Bromoxynil	455(1)	181(100)	--	

* Q=quantitation ion, C=confirmation ion

TABLE 5

Mass number (m/z) and relative abundance (%) of the $(M-181)^-$ ions observed for the PFB esters of acidic herbicides under NICI conditions

Herbicide	$(M-181)^-$		
4-CPA	185(100)	187(20)	186(18)
MCPP	213(100)	215(22)	214(19)
Dicamba	219(100)	221(66)	223(10)
MCPA	199(100)	201(20)	200(15)
2,4-DP	233(100)	235(66)	237(13)
2,3,6-TBA	223(100)	225(100)	227(39)
2,4-D	219(100)	221(66)	223(8)
Bromoxynil	276(100)	274(39)	278(55)
2,3-D	219(100)	221(60)	223(24)
Silvex	267(100)	269(98)	271(38)
2,4,5-T	255(100)	252(55)	253(43)
MCPB	227(100)	229(39)	
2,4-DB	247(100)	249(69)	251(16)
Picloram	241(100)	239(93)	

TABLE 6

Mean % recoveries and precision (standard deviations, in parenthesis) of acidic herbicides from fortified natural water samples (no. of replicates=6)

Herbicide	1 μg/L	0.1 μg/L
4-CPA	54.8(5.5)	47.6(8.4)
MCPP	84.8(5.6)	92.6(9.5)
Dicamba	66.6(3.5)	73.6(7.2)
MCPA	82.0(4.4)	73.5(5.1)
2,4-DP	92.6(4.0)	91.2(5.5)
2,3,6-TBA	50.3(2.6)	46.3(5.3)
TBA*	88.8(5.8)	89.1(7.6)
2,4-D	69.9(9.3)	73.4(9.8)
Bromoxynil	83.3(6.4)	87.1(6.3)
2,3-D	73.0(6.8)	69.2(8.8)
Silvex	93.6(3.7)	79.8(7.9)
2,4,5-T	69.5(10.5)	65.3(7.1)
MCPB	93.0(4.4)	94.5(5.5)
2,4-DB	96.9(4.5)	85.4(5.5)
Picloram	44.5(7.7)	49.2(10.1)

* An isomer of trichlorobenzoic acid with unknown structure.

FIGURE 1

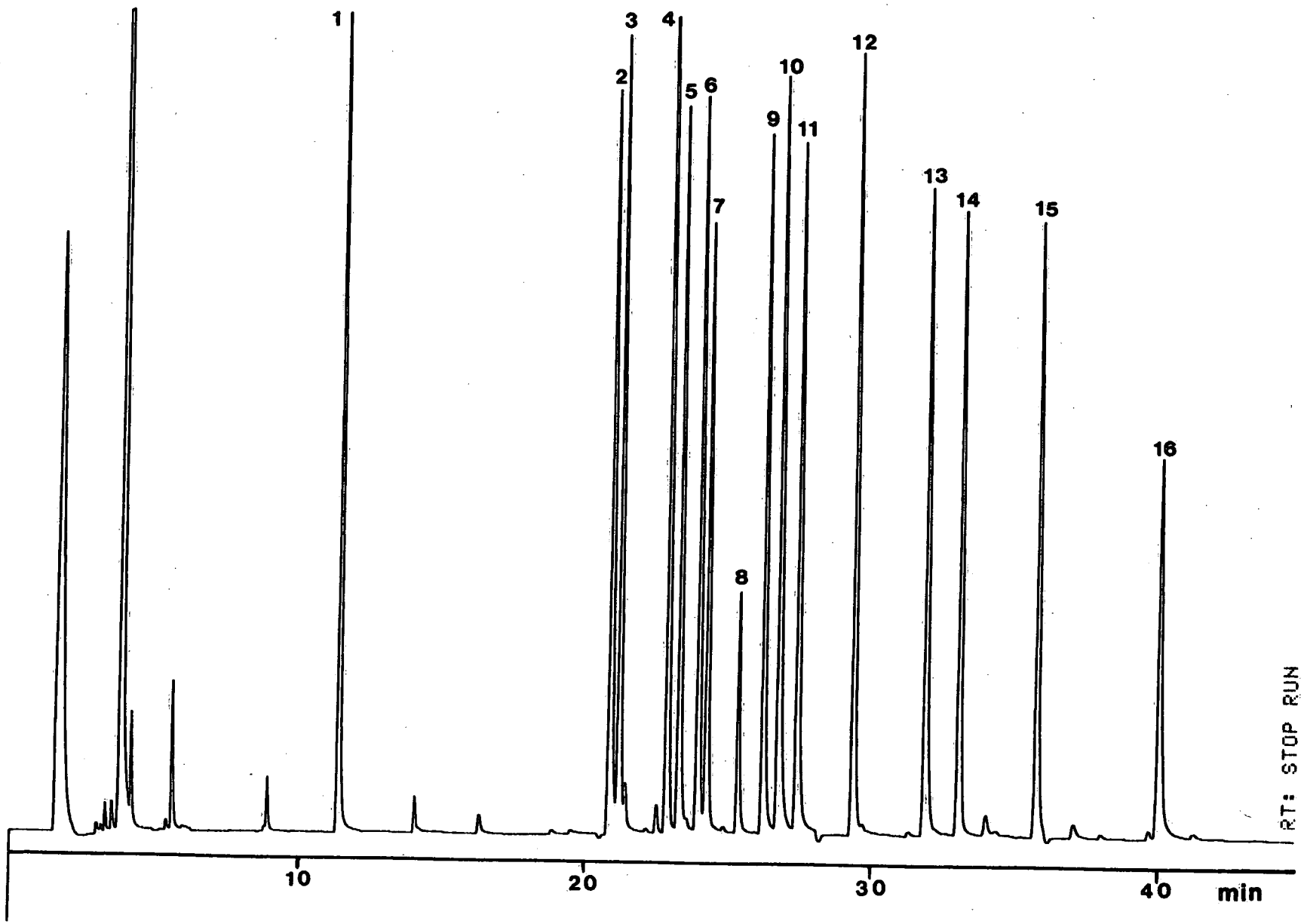


FIGURE 2

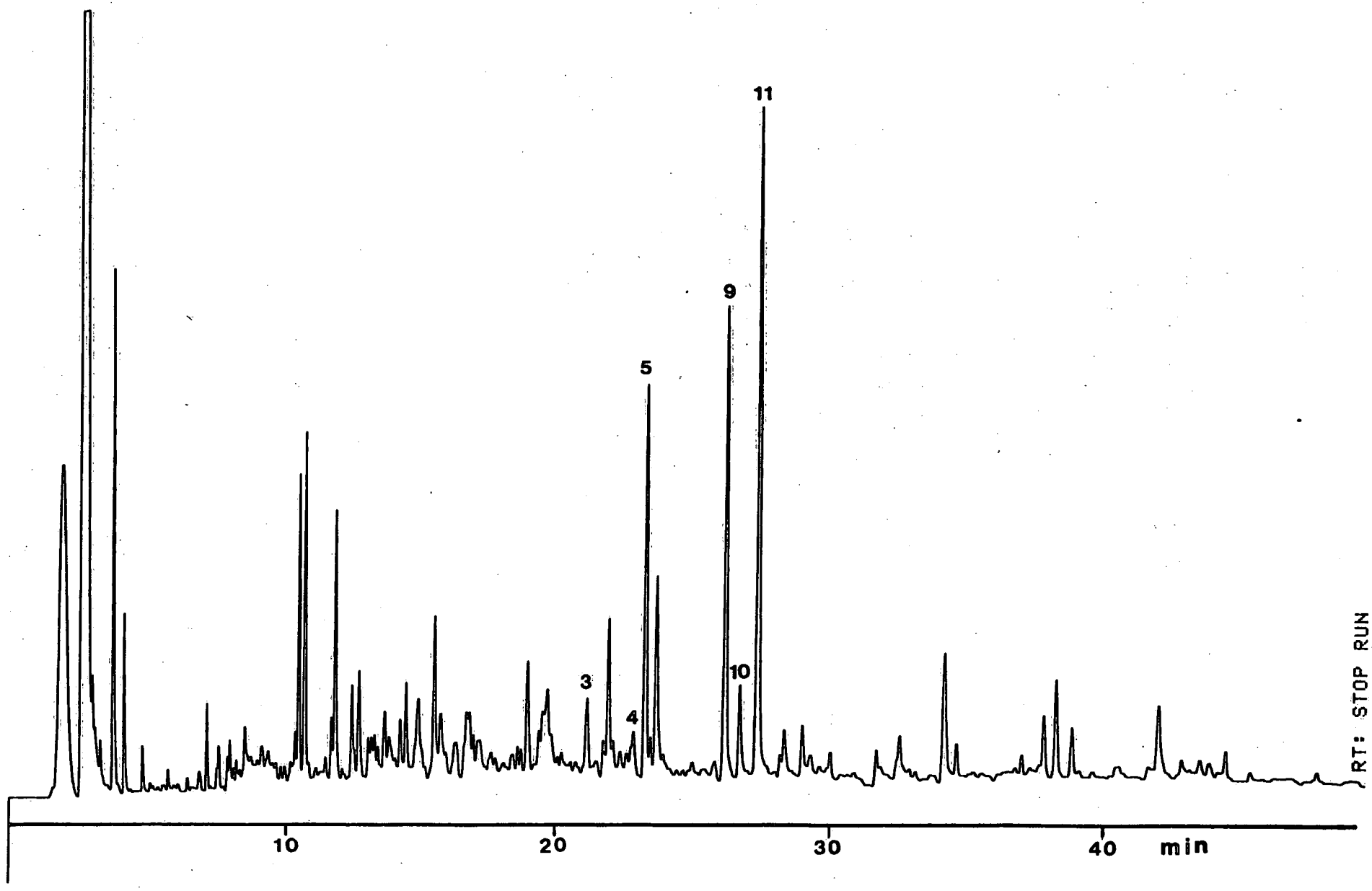
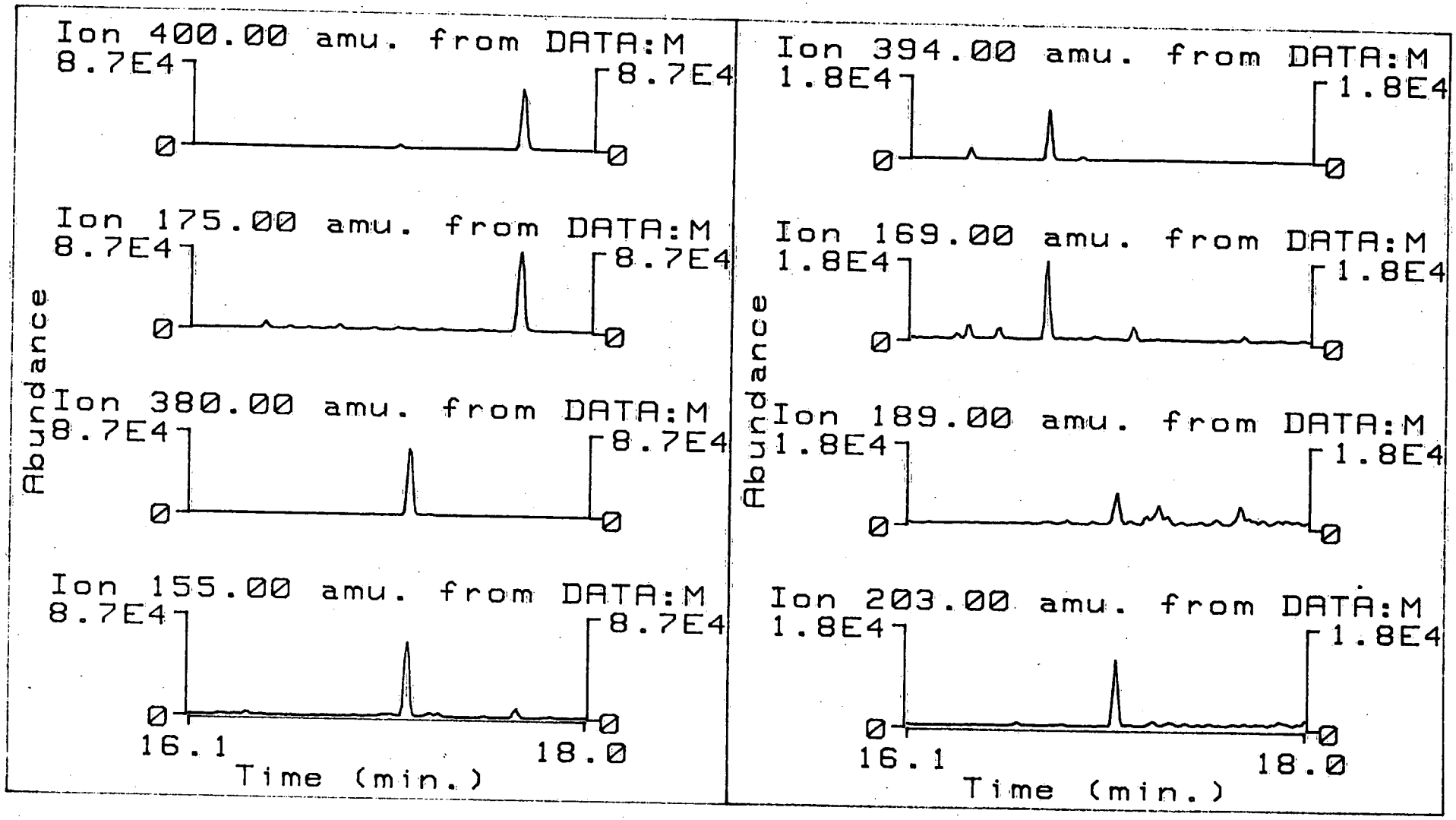


FIGURE 3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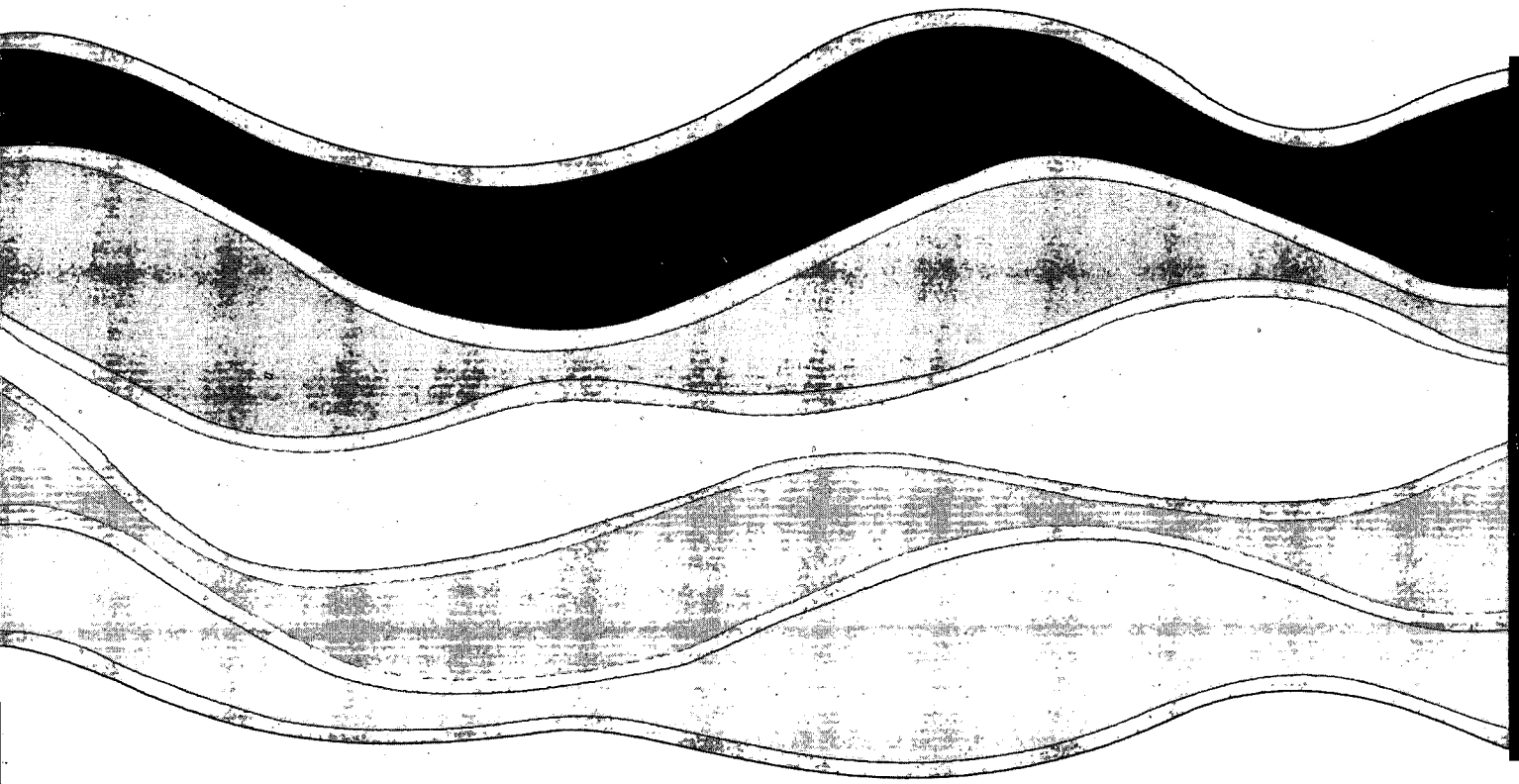
Abundance



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