Multiresidue Determination of Acidic Pesticides in Water by HPLC–DAD with Confirmation by GC–MS Using Conversion to the Methyl Ester with Trimethylsilyldiazomethane*

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Abstract

A multiresidue pesticide methodology is studied and results for acidics are reported here, with base/neutral to follow. This work studies a literature procedure as a possible general approach to many pesticides and potentially other analytes that are considered to be liquid chromatographic (LC) candidates, rather than gas chromatographic (GC) ones. The analysis of the sewage effluent of a major southwestern U.S. city serves as an example of the application of the methodology to a real sample. Recovery studies were also conducted to validate the proposed extraction step. A gradient elution program was followed for the high-performance LC (HPLC) leading to a general approach for acidics. Confirmation of identity was by electron ionization–GC–mass spectrometry (MS) after conversion of the acids to the methyl ester (or other appropriate methylation) by means of trimethylsilyldiazomethane. The 3,4-dichlorophenoxyacetic acid is used as an internal standard to monitor the reaction, and polychlorobiphenyl #19 is used for the quantitation internal standard. Although others have reported similar analyses of acids, conversion to the methyl ester is by means of diazomethane itself, rather than by the more convenient and safe trimethylsilyldiazomethane. Thus, the present paper supports the use of trimethylsilyldiazomethane with all of these acids (trimethylsilyldiazomethane has been used in environmental work with some phenoxyacetic acid herbicides) and further supports the usefulness of this reagent as a potential replacement for diazomethane. The HPLC approach here could also serve as the separation basis for an LC–MS solution for confirmation of identity, as well as quantitation.

Introduction

Under various legislative acts such as the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), the U.S. Environmental Protection Agency (EPA) has been charged with monitoring the levels of pesticides and other substances and in determining their effects on ecosystems and human health (1–5). There are now a great variety of methods for various groups of pesticides. In particular, U.S. EPA Methods 531.1 (carbamates), 631 (benomyl and carbendazim), 627 (dinitroaniline), and 632 (carbamate and urea) target various groups of pesticides for determination (6). Each group of compounds presents special problems and may also require different detection limits.

What is missing in considering all of these methods is a general approach to such analytes from the high-performance liquid chromatographic (HPLC) point of view. By this we mean an analogous methodology to that found in Methods 625 or 8270 (7), in which a large number of target analytes can be screened, and the presence of nontarget compounds can be discovered. Part of the difficulty with establishing a multiresidue analytical methodology is the lack of a universal approach to liquid chromatography (LC)–mass spectrometry (MS) for such a diverse class of compounds.

Multiresidue pesticide methods present analytical challenges in the form of great diversity of chemical structures and physical properties of the target analytes. Nevertheless, analysts have found some solutions for this complex task (8–19). Thus, with a sufficiently large subset of compounds, some fractionation of the group is probably inevitable in order to keep the separations/detections practicable.

In particular, DiCorcia and Marchetti have presented an isolation based on Carbopack (Supelco, Bellefonte, PA) solid-phase exctraction (SPE) followed by HPLC–diode-array detection (DAD) for approximately 89 compounds (8,9). Their approach divides

* The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded and performed the research described here. This work has been subjected to the Agency's peer review and has been approved as an EPA publication. The U.S. Government has the right to retain a non-exclusive, royalty-free license in and to any copyright covering this article. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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the analytes into acidic and base/neutral fractions for determination using a C_{18}-derivatized silica HPLC column with gradient elution. Because theirs is one of the largest subsets of compounds approached from a unified viewpoint, their work presents a possible fruitful approach to a general screening method for pesticides and other analytes by HPLC. An additional application of the overall approach is its potential use as a screening method in newer issues such as analysis for pharmaceuticals and personal care products (PPCPs) in effluent and other matrices (19–22). PPCPs constitute an emerging area of environmental research that encompasses a wide range of chemical structures and functionality at trace levels (23,24). Acidic target substances are obviously an important subset of PPCPs.

In addition to quantitative and qualitative identification by HPLC–DAD, a confirmatory approach is often based on a complementary separation in HPLC or is based on either gas chromatography (GC)–MS or LC–MS for greater specificity. For acidic analytes, diazomethane is often chosen to enable GC separation of esters or other methylation products in which volatility or chromatography is aided by derivatization. Trimethylsilyl diazomethane has been proposed as a safer and more convenient reagent for the derivatization (25,26).

In this work, we offer further study of a published multiresidue method for pesticides in water based on SPE and HPLC–DAD and evaluate its potential to act as a broad screening technique with the inclusion of additional analytes. In the present work, only the acidic fraction afforded by the methodology is studied. A confirmatory and quantitative determination by GC–MS is added in this work in which the application of trimethylsilyldiazomethane is further investigated for acidic analytes.

Experimental

Chemicals

The following chemicals/reagents were used. The acetonitrile was HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ). Deionized (DI) water from a Nanopure system (Waters, Milford, MA) was filtered (2-µm pore size). The methanol was HPLC-grade (Burdick and Jackson, Muskegon, MI). Trifluoroacetic acid (TFA) was 99+% and spectrophotometric grade and the sodium hydroxide was 97% and Americal Chemical Society (ACS) grade (both Aldrich Chemical, Milwaukee, WI). The hydrochloric acid was ACS grade and purchased from Mallinckrodt Baker (Paris, KY). The sodium sulfate was 99+%, granular, and ACS grade, and sodium sulfite was 98+% and ACS grade (Aldrich Chemical).

Solutions

Pesticides

Standards were obtained from EPA Pesticide Repository. The concentration of individual standard solutions was 1 mg/mL in methanol. Combined working standard was prepared by mixing 100 µL of each stock standard and diluting to 10 mL with methanol. Thirteen acidic pesticides were divided into two groups for calibration purposes. Acidic D contained dicamba, coumafuryl, 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 4-2-methyl-4-chlorophenoxybutyric acid (MCPB), and dinoseb. Acidic E contained bentazone; bromoxynil, 2,4-dichlorophenoxyacetic acid (2,4-D), mecoprop, 2,4-dichlorophenoxy-2-butyric acid (2,4-DB), 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP), and pentachlorophenol (PCP).

Mobile phase HPLC

The HPLC mobile phase consisted of methanol–acetonitrile (820 mL methanol combined with 180 mL acetonitrile) and water (1.7 mL of TFA added to 998.3 mL water).

6N NaOH

An amount of 25.2 g of NaOH was dissolved in water and diluted to 100 mL.

Sample collection and treatment

Three 4-L samples were collected from the sewage effluent. The contents of each 4-L bottle were dealt with in parallel fashion following identical procedures. To isolate base/neutral substances, each 4-L sample was basified to pH 12–14 with 6N aqueous NaOH. After basification, each 4-L sample was extracted three times with 400 mL dichloromethane. The aqueous phases were then taken to pH 1 with concentrated hydrochloric acid and again extracted three times with dichloromethane. The dichloromethane extracts from these acidified solutions were combined, dried over anhydrous sodium sulfate, and concentrated to a volume of 2.4 mL. Concentration was achieved using a refluxing apparatus consisting of a large round-bottom flask, boiling chips (Teflon), and a three-ball Snyder column; this ensured that all surfaces were continuously bathed with condensing liquid during concentration. A 240-µL (10%) aliquot of the concentrate was subjected to GC–MS and HPLC analysis.

SPE procedures

A 1-L separatory funnel, 7-cm short stem funnel, 1-L side arm filtering flask, and vacuum pump were used (all Gast, Ann Arbor, MI). Also used was a Visiprep SPE vacuum manifold (Supelco).

The SPE cartridge used was a 250-mg (6 mL) Envi-Carb of graphitized nonporous carbon with a surface area of 100 m^2/g and 120/400 mesh (Supelco).

The cartridge was washed with 5 mL of methylene chloride (80:20, v/v), followed by 2 mL of methanol, air dried 1 min, and then rinsed with three 5-mL volumes of 10 g/L ascorbic acid in HCl acidified water (pH 2). The cartridge was not allowed to dry during the final rinses, and 4 mL of final solution remained in the tube before starting sample extraction (vacuum pump restarted).

One liter of DI water containing 0.2 g of sodium sulfite and fortified with known standards including an internal standard was mixed in a separatory funnel. Water from the separatory funnel was forced through a 7-cm short stem funnel and the cartridge at 60 mL/min. Just after the sample was passed through the cartridge, the sides of the funnels were washed down with DI water to remove traces of aqueous sample. The upper frit was pushed against the carbon bed. Pressure was reduced with air drying to remove all traces of water. The cartridge was moved to the Visiprep SPE vacuum manifold. A round-bottom test tube (1.4 × 12.5 cm) was located below the tube; an unwanted fraction was removed by passing through 1 mL of methanol, drop wise, the

last drops of methanol removed by reducing the pressure.

The acidic pesticides were collected in a second tube by drawing through the cartridge 3- × 4-mL aliquots of methylene chloride–methanol (80:20, v/v) acidified with TFA (0.2%, v/v). This solution was stored in the freezer when not being used and was prepared fresh every other day. The pressure was reduced to remove the last drops of eluant. Before blow down, the fraction was neutralized with 50 µL of water–methanol solution of ammonia mix (2 mL of concentrated ammonia diluted to 10 mL with methanol) by vortexing. The sample tube was placed in a water bath for evaporation to dryness at 30ºC under a gentle stream of nitrogen. The sample was reconstituted with 300 µL of water–methanol (60:40, v/v) acidified with TFA (0.05:5, v/v), vortexed, and sonicated for 20 min.

Recovery studies of acidic pesticides

Samples of 1 L of DI water were fortified with 0.5 and 1.0 µg of combined pesticides and then extracted according to the previous setup.

Derivatization with trimethylsilyldiazomethane

Derivatizations with trimethylsilyldiazomethane were carried out in commercially silanized (via high-temperature treatment with hexamethyldisilazane) vials (12- × 32-mm wide-mouth screw cap with polytetrafluoroethylene/silicone cap liners). These vials were purchased from Alltech (Deerfield, IL).

For calibration purposes, the vials were charged with an acetone solution containing varying amounts of a stock solution of 2,4-D and 2,4,5-T in acetone (0.0506 g in 100 mL) so that the herbicide concentrations in a 1-mL total volume systematically varied for calibration purposes. A total of seven levels were employed. On top of the 1.0 mL in each vial was added 50 µL of a stock solution of 3,4-D in acetone (0.0186 g in 100 mL) as an internal standard, 25 µL of the 2.0M trimethylsilyldiazomethane reagent, and 100 µL of methanol. The 1 mL of extract in methanol recovered from the sewage effluent was treated in the same way. After thorough mixing, the homogenous reaction mixtures were allowed to stand at ambient temperature for 2 h.

GC–MS analysis

GC–MS analysis was carried out directly on the reaction vial contents on an Agilent Technologies 6890 GC/5973 mass selective detector (MSD) (Palo Alto, CA). A 30-m × 0.25-mm i.d. HP 5MS column with a 0.25-µm film was used with the MSD. The temperature program was 46.0-min long and ramped as follows: 60ºC to 150ºC at 10.00ºC/min for 9 min, 150ºC to 250ºC at 4.00ºC/min for 25 min, 250ºC to 300ºC at 10.00ºC/min for 5 min, and maintained at 300ºC until 46.00 min total was reached.

Injections were 2 µL and pulsed; the splitless mode was used. The carrier gas was He at a flow rate of 1.0 mL/min with pressure programming, and the instrument was operated in electron impact (EI) mode. The retention times of 2,4-D and 2,4,5-T methyl ester were 11.70 and 14.48 min, respectively. The ion masses monitored (dwell time 50 ms, resulting electron microscopy = 2035.3 V) were, respectively, 2,4-D and 2,4,5-T methyl ester at *m/z* 234.0, 236.0, and 219.0; 2,4,6-T at *m/z* 268.0, 270.0, and 253.0; and polychlorobiphenyl (PCB)#104 *m/z* 325.9.

GC–MS analysis was carried out directly on the reaction vial contents on a VG 70SE. A 30-m × 0.25-mm i.d. DB 5MS column (J&W Scientific, Folsom, CA) with a 0.25-µm film was used. The temperature program was the same as for the MSD.

Injections were 2 µL using an oncolumn injection mode. The carrier gas was He at a flow of 30 cm/s, and the instrument was operated in EI mode. The retention times of the methyl esters of 2,4-D and 2,4,5-T were 13:20 and 16:10 min:s, respectively. The ion masses monitored (dwell time, 50 ms; settling time, 30 ms; photomultiplier setting, 410) were, respectively: 2,4-D and 2,4,5-T methyl ester at *m/z* 233.9850 and 235.9821, 2,4,5-T methyl ester at *m/z* 267.9461 and 269.9431, and PCB #19 at *m/z* 257.9584.

Recovery levels from effluent spiking studies

Recoveries were assessed from spiking studies carried out as follow. One-liter samples of DI water were fortified with a level of 1 µg/L of 2,4-D and 2,4,5-T and processed by SPE.

For GC–MS analysis, eluent was evaporated to dryness and then subjected to partitioning between methylene chloride and water (acidified with HCl). The methylene chloride fraction was then dried over sodium sulfate and subjected to the derivatization procedure.

HPLC separations

Beckman System Gold software (Beckman, Fullerton, CA) with a Beckman Model 126 pump unit HPLC system was used. An LC_{18} with 5-µm packing in a 150- × 2.00-mm Luna column (Phenomenex, Santa Clara, CA) was also used. Acidic pesticides were chromatographed with methanol–acetonitrile (82:18, v/v) and water acidified with TFA (0.17%, v/v). The initial mobile phase consisted of organic–acidified water (50:50) and was linearly increased to 88% organic after 35 min, held for 5 min, 5 min to 50% organic, and equilibrated for 12 min. The flow rate was 0.2 mL/min, and the UV detector was set at 230 nm.

Results and Discussion

Recoveries

Table I tabulates results for two spiking levels (0.5 and 1.0 µg/L) for the 13 acidic analytes. Generally, the recoveries were quantita-

Table I. Recoveries of 13 Acidic Analytes by SPE

Analyte	0.5-ppb level	1.0-ppb level
Bentazon	74.2 ± 14.6 %	57.6 ± 14.5 %
Dicamba	110.2 ± 4.5 %	100.1 ± 8.7 %
Bromoxynil	86.6 ± 4.4 %	88.6 ± 6.7 %
Coumafuryl	85.8 ± 64.7 %	63.3 ± 32.1 %
2,4-D	88.5 ± 6.2 %	94.6 ± 7.6 %
MCPA	93.2 ± 14.7 %	80.6 ± 8.0 %
Mecoprop	62.9 ± 11.4 %	71.6 ± 17.0 %
2,4,5-T	111.2 ± 5.0 %	91.5 ± 7.1 %
2,4-DB	60.4 ± 10.5 %	64.6 ± 26.3 %
MCPB	62.6 ± 11.2 %	64.0 ± 3.0 %
2,4,5-TP	124.3 ± 5.7 %	126.7 ± 9.6 %
Dinoseb	100.1 ± 8.3 %	99.5 ± 10.6 %
PCP	58.6 ± 19.3 %	58.4 ± 30.0 %
Overall average	both levels	83.2

tive, ranging from 57.6% to 126.7% with an overall average recovery of 83.2%. The values in the table represent either four or five separate determinations. The standard deviations occasionally exceeded 20%, and for those compounds, some care in the interpretation of the quantitations should be taken. The referenced work obtained recoveries above 90%, consistently. Although our results were not quite as good, they were comparable to results reported in the original work, lending evidence that the methodology is practical for adoption by other laboratories.

Because one of our stated goals was to test the methodology for applicability to a broad range of analytes, these data support the method's ability to recover multiple residues. This, then, opens the possibility of applying the approach to an even broader range of compounds in order to establish its ability to act as a general screening tool consisting of preconcentration by SPE and HPLC–DAD–UV.

To add support to a broader range of compound applicability, we obtained recoveries for salicylic acid (analgesic metabolite), trichloropyridinol (pesticide metabolite), and clofibric acid (lipid regulator metabolite). Average recoveries were 85% ± 15% at the 1-µg/L fortification level. These data indicate that the SPE followed by HPLC–DAD performs well for compounds and compound classes other than the original acidic (and base/neutral) pesticides.

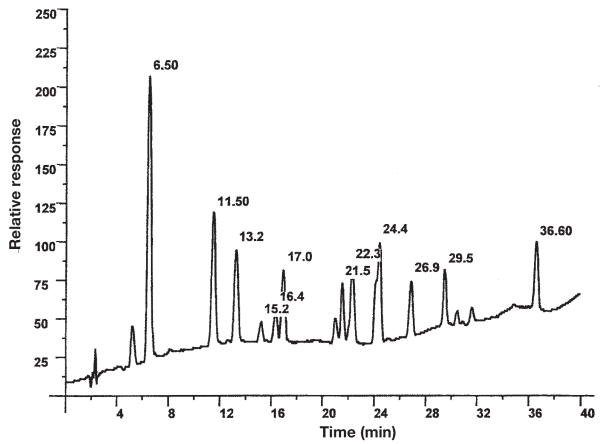

Figure 1. HPLC separation of 13 acidic analytes with retention time (min): 2-chlorobenzoic acid (internal standard) (6.50), dicamba and bentazone (11.5), bromoxynil (13.2), coumafuryl (15.2), MCPA (16.4), 2,4-D (17.0), mecoprop (21.5), 2,4,5-T (22.3), 2,4-DB and MCPB (24.4), 2,4,5-TP (26.9), dinoseb (29.5), and PCP (36.6).

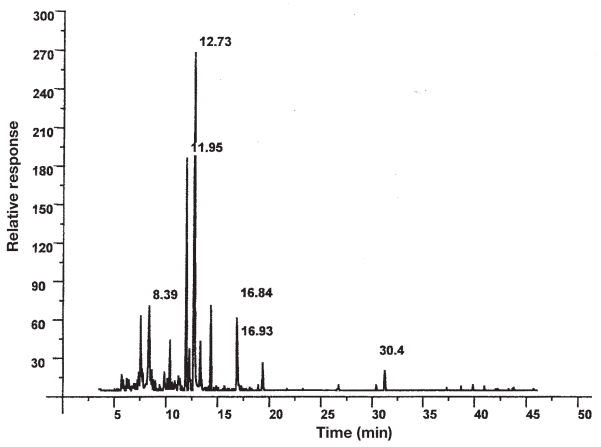

Figure 2. GC–MS full-scan total-ion chromatograms of acidic D-analytes as methyl esters with retention time (min): dicamba (11.95), coumafuryl (30.4), MCPA (12.73), 2,4,5-T (16.93), MCPB (16.84), and dinoseb (18.96).

HPLC separation

Our approach differs from the original work in using a 2-mm-i.d. HPLC column. This diameter column presents a compromise between the standard analytical column (4.6-mm i.d.) in terms of concentration detection limits (~ a factor of 2 higher with the narrow bore) and reducing solvent usage (reduced to 19% of original), while maintaining full compatibility with potential LC–MS analysis (200-µL/min flow rate when using atmospheric pressure chemical ionization interface). According to Figure 1, we did not achieve full baseline separation of all 13 compounds with this column. Incomplete resolution of bentazon and dicamba, as well as 2,4-DB and MCPB, was obtained in our laboratory, as well as in the original work. Thus, recovery studies were carried out in two separate groups.

This incomplete separation gives us some measure of the relative selectivity of the method for these kinds of acids, and we can reasonably expect that coelutions are going to occur in real, complex samples.

Naturally, no one environmental sample is expected to contain all 13 compounds but may of course contain a number of coextractives that could interfere with determinations. Thus, confirmation of identity and comparative quantitation must be provided to the screening procedure for completeness. Generally, this is provided by GC–MS (with derivatization) or LC–MS, although the original work depended on a second column with HPLC–DAD (4).

GC–MS

In the present study, confirmation was obtained by GC–MS under EI conditions for the methyl esters or other methylation product of the analytes obtained from reaction with trimethylsi-

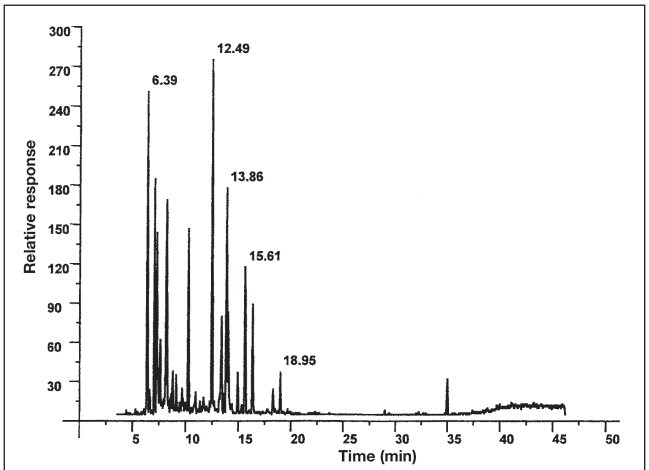

Figure 3. GC–MS full-scan total-ion chromatograms of acidic E-analytes as methyl esters with retention time (min): bentazone (18.95), bromoxynil (13.86), 2,4-D (14.01), mecoprop (12.49), 2,4-DB (18.35), 2,4,5-TP (10.87), and PCP (15.61).

lyldiazomethane. A capillary GC on DB5 failed to separate all 13 components, as did HPLC (Figure 2). The pair 2,4,5-T and MCPB were not resolved (~ 0.03-min separation), and bromoxynil and 2,4-D were just resolved (0.16 min). In addition, dinoseb is only slightly resolved from 2,4-DB (0.06 min). However, unique monitoring masses for each compound were selected for confirmation and quantitation.

Figures 2 and 3 present the separation of the compounds in the two recovery groups used. Dinoseb gave a very weak response within the first group near 10.3 min. The 3,4-D isomer was originally chosen as internal standard to monitor the completeness of the reaction, as well as exhibit quantitation. The use of PCB non-Aroclor congeners was eventually selected as the main and general quantitation agent for this, as well as other residue determinations. We present the application of GC–MS methodology as a confirmatory tool in this work with quantitative data presented for all analytes as a demonstration that the approach is also appropriate for quantitation. In addition, further study of separations of target analytes on different GC phases could be conducted in search of more complete separations. However, we have chosen to present the work on the most commonly used GC column in environmental work. An approach based on LC–MS is also feasible and warrants further study using the separation presented (or modified).

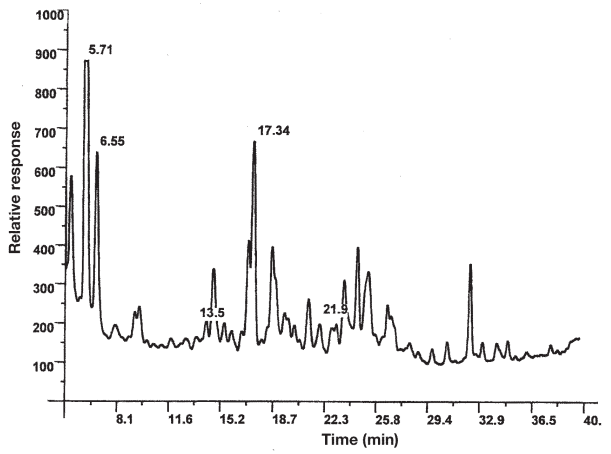

Figure 4. HPLC of extract of effluent in which responses at retention times 13.5 and 21.9 represent presumptive amounts of 0.3 and 0.4 µg/L of bromoxynil and mecoprop in the effluent (unconfirmed by GC–MS).

Table II. Amounts of Herbicides and Other Acidics in Runoff Water* Reported in ng Isolated From 500 mL Runoff Water Each Sampling Day†

Compound	Day 1	Day 4	Day 7	Day 12	Day 14
Dicamba ME	65.1	272	109	105	122
Bentazone ME	1297	657	106	200	162
2,4,5-TME	3966	2577	830	757	795
DinosebME	2230	1661	284	286	294
MCPAME	3087	3016	1158	1030	991
2,4,5TPME	4050	3120	963	781	647
MCPBME	2916	967	173	82.4	362
BromoxynilME	5714	3152	722	466	422
2,4-DME	4920	4212	1309	61.0	68
MecopropME	5025	3315	1098	844	911
2,4-DBME	4072	1752	295	122	123
PCPME	6124	976	312	266	239
Methyl salicylate	17.9	11.0	24.0	8.06	9.47
Methyl clofibrate	66.7	59.3	9.91	38.5	27.9
TCP methyl ester	570	439	400	95.3	93.6
Coumofuryl ME	226	156	146	148.6	175.6

* 20-µg total application for each compound.
† Levels below 300 ng are not confirmed.

Sample analysis

Unfortified and fortified drinking water and sewage effluent were examined as practical applications of the HPLC screening

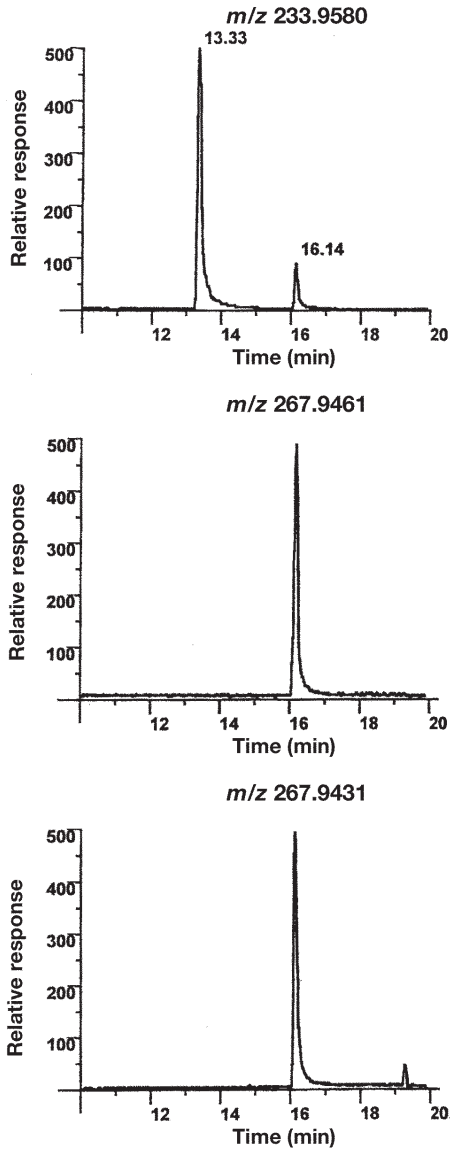

Figure 5. GC–MS-selected ion recording results for 2,4,5-T as the methyl ester confirmed in effluent spiked at 1.0 µg/L and quantitated at 0.85 µg/L.

methodology. Figure 4 presents a chromatogram for the preconcentrated effluent using the traditional liquid–liquid extraction technique as part of a broad characterization effort. Tentative peaks for pesticides were detected at < 1 µg/L at retention times of 13.5 and 21.9 min. None of these were confirmed. A very weak response at the retention time of clofibric acid was observed, and this analyte has been the subject of a separate paper in which clofibric acid was confirmed in the extract of effluent (23). The chromatogram suggests the robustness of the separation and the possibility of detecting analytes below 1 µg/L in complex matrices such as effluent.

Effluent was spiked at 1 µg/L in 2,4-D and 2,4,5-T, and the sample was subjected to SPE. The effluent sample afforded by SPE directly caused immediate vigorous bubbling with the addition of derivatizing agent. To overcome coextractive effects and competition from TFA, the sample eluent was evaporated to dryness and then subjected to partitioning between methylene chloride and water (acidified with HCl). The methylene chloride fraction was then dried over sodium sulfate and subjected to the derivatization procedure. Figure 5 shows the result of confirmation of 2,4,5-T as the methyl ester, which was quantitated at 85% recovery (850 ppt). Ions at *m/z* 234, 268, and 270 are shown. The 2,4-D spike was recovered at 114% as the methyl ester. Thus, the feasibility of using GC–MS with derivatization for confirmation of identity and quantitation is shown.

An additional test and application of the GC–MS methodology was undertaken. The attenuation of herbicides determined in runoff water was studied using an experimental test plot. After spraying the plot with herbicides, the area was watered and runoff water analyzed over a period of 14 days. These determinations were performed using the GC–MS methodology as a test of its robustness. The results are tabulated in Table II.

Some of the compounds were almost immediately dissipated, presumably either through decomposition or irreversible adsorption. Others could be followed in a descending presence in runoff water. In some cases, partial interferences were observed on some ions. These issues point out the problems with environmental monitoring of herbicides. The herbicides frequently dissipate rapidly, the application rates may be relatively low, and the sampling events must be closely tied to the application events. Interferences are a major issue with low-level detection, and the development of efficient class separations as cleanup for complex extracts containing these analytes remains a future research goal.

Conclusion

The present work further explored a multiresidue method for pesticides (acid fraction) with the determination of target compounds to less than the 1-µg/L level. This methodology is proposed as a general approach to the analysis of pesticides by HPLC following acid and base/neutral partitioning. At the same time, we presented a confirmatory/quantitation technique for acids based on derivatization with trimethylsilyldiazomethane followed by GC–MS and demonstrated its effectiveness at levels below µg/L. The SPE–HPLC–DAD approach appears to be broadly applicable and was demonstrated to work with both pesticide and pharmaceutical metabolites. This suggests that the methodology applies to a broad spectrum of analytes with the ability to detect nontargeted compounds. Future work will address the separations and confirmation of the base/neutral fraction, as well as effective cleanup approaches.

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Manuscript accepted June 4, 2003.