

Analysis of Some Chlorophenoxy Acids and Carbamate Herbicides in Water and Soil as Amide Derivatives Using Gas Chromatography–Mass Spectrometry

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Abstract

A newly developed method for determining three phenoxy acids and one carbamate herbicide in water and soil samples using gas chromatography with mass spectrometric detection is developed. Phenoxy acids are derivatized through a condensation reaction with a suitable aromatic amine. 1,1-Carbonyldiimidazole is used as a condensation reagent. Derivatization conditions are optimized with respect to the amount of analyte, amine, solvent, and derivatization reagent. The optimum derivatization yield is accomplished in acetonitrile. 4-Methoxy aniline is used as a derivatizing agent. Obtained derivatives are stable indefinitely. Enhancement in sensitivity is achieved by using the single-ion monitoring mass spectrometric mode. The effectiveness of the developed method is tested by determining investigated compounds in water and soil samples. Analytes are concentrated from water samples using liquid-phase extraction and solid-phase extraction. Soil samples are extracted using methanol. Detection limits of 1.00, 50.00, 100.00, and 1.00 ng/mL are obtained for 2-(1-methylethoxy)phenyl methylcarbamate (Baygon), 2-(3-chlorophenoxy)-propionic acid (Cloprop), 2,4,5-trichlorophenoxyacetic acid, and 4-(2,4-dichlorophenoxy)butyric acid, respectively. LPE for spiked water samples yields recoveries in the range of 60.6–95.7%, with relative standard deviation (RSD) values of 1.07–7.85% using single component calibration curves. Recoveries of 44.8–275.5%, with RSD values ranging from 1.43% to 8.61% were obtained using a mixed component calibration curves. SPE from water samples and soil samples showed low recoveries. The reason is attributed to the weak sorption capabilities of soil and Al₂O₃.

Introduction

Phenoxy acid and carbamate herbicides are phytotoxic compounds widely used in agriculture. They are common in soil, as well as surface and ground waters (1). Some of these herbicides are on the priority list of pollutants in many countries all over the world (2). 4-(2,4-Dichlorophenoxy)butyric acid (2,4-DB)

and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are endocrine disrupter chemicals (3), and because of their high toxicity and degradation possibility into chlorinated phenols, these herbicides should be monitored in aquatic and soil environments (4).

Phenoxy acids are ionic compounds with a low separation efficiency on reversed-phase (RP) high-performance liquid chromatography and gas chromatography (GC) nonpolar stationary columns because of exposed siloxane groups, which permit a secondary interaction. The GC analysis of these compounds is also restricted by its low volatilities because of the hydrogen bonding between carboxylic acid and phenol functionalities. These functional groups can cause the parent molecules to adsorb on the column, producing asymmetric peaks. Therefore, an effective derivatization of these polar groups can significantly increase the volatility and reduce the adsorption effects of these compounds. Solid-phase extraction (SPE), solid-phase microextraction (SPME), and liquid-phase extraction (LPE) have been used for concentrating some of these herbicides from water samples (5–12).

Several derivatization methods were reported for the GC–mass spectrometry (MS) analysis of phenoxy acid herbicides. Silylation and alkylation are the usual derivatization procedures. Because silyl derivatives are easily hydrolyzed in the presence of moisture, a combination of silylation and SPE is not feasible. Alkylation using diazomethane, boron trifluoride, methanol, or pentafluorobenzyl bromide (PFB) was reported (13). Diazomethane has been frequently used as a derivatizing reagent. It is an extremely toxic, highly irritating, and explosive compound that needs an excessive cleanup process when used (6,8). Methylation with boron trifluoride methanol can not be combined readily with SPE, also PFB esters are unstable. Tetra-alkylammonium salts were used to improve the extraction efficiency of these compounds and to achieve their further derivatization in the hot injector of the GC (9,14). Other derivatization reagents, such as alkyl chloroformates, have been reported (7). In these cases, extracts had to be evaporated to dryness before derivatization could take place, and the excess reagent had to be cleaned-up after completing the reaction. As a consequence, such derivatizations are time-consuming and hazardous processes.

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Less polar compounds amenable for GC analysis, without the need of removing the excess derivatizing reagent or the reaction byproducts, were obtained by silylation (15–17). 2-Cyanoethyl-dimethyl-diethylamino-silane (18) and *N,O*-bis(trimethylsilyl) trifluoroacetamide were used to transform several phenoxy acid compounds into their trimethylsilyl derivatives (19). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide was used as a silylation reagent for phenoxy acids extracted from water samples using SPE into *tert*-butyldimethyl-silyl derivatives (20). However, the silylation of phenoxy acid herbicides have been not recommended because the byproducts of the reaction led to a noisy GC baseline, resulting in an increase of the detection limits of the method (21).

Carbamates derivatized by flash methylation were determined using a GC–MS–MS system with a PolarisQ external source ion-trap MS operated in electron impact (EI)–MS–MS (22). Nine phenoxy acid herbicides from soil, pond water, and lawn clipping matrices were methyl derivatized and determined using the same system (23). Phenoxy acid herbicides in soil samples were extracted into a diethyl ether–water solvent mixture, and they were derivatized into their methyl derivatives using methanol in the presence of H_2SO_4 as a catalyst. Resultant methyl derivatives were determined by a GC–flame-ionization detector and GC–MS (24).

The aim of this investigation was to evaluate the feasibility of a new derivatization procedure based on the amide formation using carbonyl diimidazole as a coupling reagent and 4-methoxy aniline as a derivatizing agent. Amide products have high thermal and hydrolytic stabilities. Analytes extracted from water using LPE or SPE and from soil samples were derivatized and determined using GC–MS detection. The influences of experimental factors, such as time, solvent, derivatizing agent, and the quantity of carbonyl diimidazole on the derivatization yield, were investigated. Derivatives were characterized using C, H, and N analyses, infrared (IR), nuclear magnetic resonance, and GC–MS. The recovery of the developed procedures was evaluated using spiked water and soil samples. The sensitivity and precision were evaluated.

Experimental

Reagents and materials

Phenoxy acid herbicides, including 2-(3-chlorophenoxy)-propionic acid (Cloprop), 98% purity; 2,4,5-T, 98% purity; 2,4-DB, 95% purity; and 2-(1-methylethoxy) phenyl methyl-

carbamate (Baygon), 99% purity were purchased from Aldrich (Milwaukee, WI). The structures of these compounds are given in Figure 1.

The derivatizing agent, 4-methoxy-aniline, was obtained from Aldrich. Carbonyl diimidazole was purchased from Sigma (Steinheim, Germany). High-performance liquid chromatography grade methanol and acetonitrile were obtained from Riedel-de Flaen (Seelze, Germany). Ethylacetate was supplied by Merck (Darmstadt, Germany). Individual and mixed component stock solutions of the four investigated herbicides were prepared in acetonitrile. Diluted standards prepared in acetonitrile were used to spike water samples. Chromatographic Al_2O_3 , obtained from BDH (Poole, UK), was used for SPE.

Equipment

GC–MS measurements were performed using a Finnigan-Trace GC 2000 (Thermo Quest, Strada Rivoltana, Milan, Italy) equipped with a split-splitless injector, AS-3000 autosampler (Thermolectron, Strada Rivoltana), and a quadropole MS (Trace-

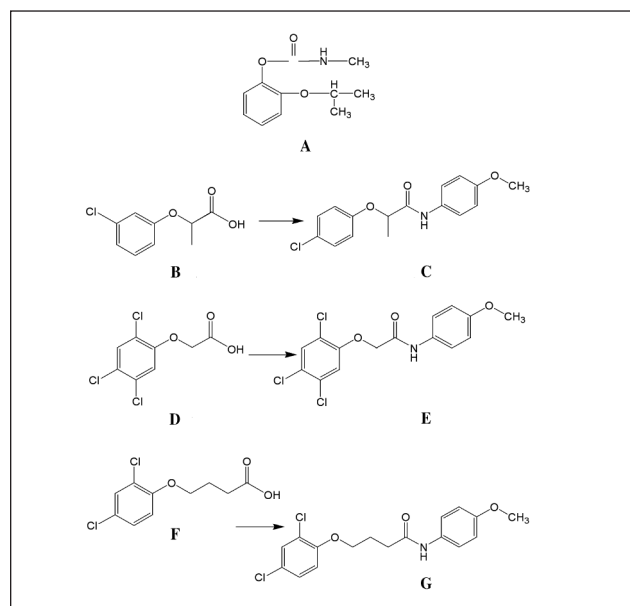


Figure 1. Structural formula of underivatized and derivatized phenoxy acids and carbamate herbicides: 2-(1-methylethoxy) phenyl methyl carbamate (Baygon) (A); 2-(3-chlorophenoxy)-propionic acid (Cloprop) (B); *N*-(4-methoxy-phenyl)-amide derivative of Cloprop (C); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (D); *N*-(4-methoxy-phenyl)-amide derivative of 2,4,5-T (E); 4-(2,4-dichloro-phenoxy) butyric acid (2,4-DB) (F); and *N*-(4-methoxy-phenyl)-amide derivative of 2,4-DB (G).

Table I. Elemental Analyses of Derivatized Phenoxy Acids and Carbamate Herbicides

Original herbicide	Derivative compound	C%		H%		N%	
		Calc.	Found	Calc.	Found	Calc.	Found
Baygon	Methyl-carbamic acid-2-isopropoxy-phenyl-ester	63.14	63.80	7.23	7.72	6.69	6.83
Cloprop	1-Chloro-4-isopropoxy-benzene- <i>N</i> -(4-methoxy-phenyl)-formamide	63.45	63.70	6.26	5.96	4.35	4.53
2,4,5-T	2,4,5 Trichloro-5-methoxy-benzene- <i>N</i> -(4-methoxy-phenyl)-acetamide	51.02	50.40	4.28	3.22	3.72	3.32
2,4-DB	4-(2,4-Dichlorophenoxy)- <i>N</i> -(4-methoxy-phenyl)-formamide	57.64	57.60	4.84	4.72	3.95	4.57

MS Finnigan, Wythenshawe, Manchester, UK) detector with a mass range of 1 to 1022 amu for detecting derivatized phenoxy herbicides. Separations were carried out using an RTX-5-MS (30 m × 0.25 mm × 0.25 mm) capillary column thermally stable to 350°C (Restek, Bellefonte, PA). Helium (99.999%) was used as a carrier gas at a constant flow of 1.6 mL/min. A RJ-45 diode array single beam UV-vis spectrophotometer (Agilent Technologies, Vienna, Austria), matched with 1 cm quartz or silica cells, was used for absorption measurements.

The IR spectra were measured using a Thermo Nicolet, NEXUS-470 FT-IR spectrometer. A Buchi rotavapor R-200, supported with Buchi heating bath B-490 (Buchi, Flawil, Switzerland), was utilized for solvent evaporations. ¹H-NMR measurements were carried out using a Varian 200 MHz, Mercury-200 NMR spectrometer operating at field a magnetic field strength of 4.7 T.

Procedures

Derivatization

Phenoxy acid herbicide amide derivatives were prepared by mixing equimolar amounts (0.001 moles) of each investigated phenoxy acid with carbonyl diimidazole in acetonitrile. The mixture was stirred at room temperature for 30 min. An equimolar amount (0.001 mole) of 4-methoxy-aniline was added, and the mixture was stirred and refluxed for 1 h at 80°C. The solution was cooled at 5°C in a refrigerator overnight. Resultant precipitated amides were filtered, washed, recrystallized, and stored at room temperature. They were subjected to an elemental analysis, IR, and NMR analyses, and the amides served as primary standards. An elemental analysis was performed using a C, H, N analyzer (Thermofinnigan 11120) at the University Central Laboratory Unit (Table I).

Measurement parameters

GC injections were performed in the splitless mode with a purge time of 1.0 min and an injection volume of 1.0 µL. The GC oven was programmed as follows: 2 min at 50°C; first ramp at 10°C/min to 150°C; second ramp at 15°C/min to

240°C; and held for 30 min. The GC-MS interface was set at 260°C. The quadrupole MS detector was set at 250°C. Mass spectra were obtained in the EI mode using a 70 eV electron beam in the full scan or single-ion monitoring (SIM) modes. A mass range between 100 and 400 *m/z* was initially monitored using the full scan mode. The SIM mode, based on the most significant 10 fragmented masses of derivatized compounds, was used for all quantitation processes. Chromatograms were collected after 4 min when all the solvent was swept out of the column. The retention times and *m/z* ratio used for quantitative purposes are given in Table II.

Standard solutions

Standard solutions having concentrations of 0.001–50 µg/mL of each pesticide were prepared in acetonitrile. A parallel series of mixed component standard solutions of the four compounds, having concentrations of 0.001–50 µg/mL in each pesticide, were prepared. These standards were injected into the GC-MS and measured under the conditions previously described. Results were evaluated using the X-Calibur software. The areas of the peaks for each compound from a single component and mixed component standards were plotted versus its ppm concentrations on a log-log scale. Two calibration graph sets were obtained for each compound.

Water treatment: LPE

A 100-mL tap water sample was sprayed with a mixture of the four herbicides dissolved in acetonitrile at a concentration of 0.001M in each investigated herbicide to yield the concentrations listed in Table III. A 50-mL portion of diethyl ether was added. The solution was shaken well, and the organic phase was separated using a separatory funnel. A second 50 mL of diethyl ether was added to the aqueous phase, shaken well, and separated. Ether fractions were collected, dried over anhydrous CaCl₂, filtered, and evaporated to dryness. Phenoxy acid residues were collected into 50 mL of acetonitrile and derivatized by adding an equimolar amount of carbonyl diimidazole, stirring for 30 min, then adding 0.003 moles of methoxy aniline, as previously described. A mixed solution containing

derivatized products was transferred into a volumetric flask and increased to 1000 mL using acetonitrile.

The resulting solution was subjected to GC-MS analysis under the conditions previously given. Recoveries were determined using the single and mixed component standard solution calibration curves.

Water treatment: SPE

A 100-mL tap water sample, sprayed with a mixture of the four herbicides dissolved in acetonitrile at a concentration of 0.001M in each investigated herbicide, and yielding the concentrations listed in Table III, was passed through a 30.0-cm length and 3.0-cm diameter column filled with approximately 50–55 g of chro-

Table II. Molecular Weights, Retention Times, Base Peaks, and the Most Intense MS Fragments of the Derivatized Phenoxy Acid Herbicides Obtained in the Electron Impact Mode Using 70 eV Electron Beam

Compound	Molecular weight (g/mol)	Retention time	Base peak cluster (<i>m/z</i> ratio) quantitation ions	<i>m/z</i> Ratio for other fragments
Baygon	209.24	14.74	109.5–110.8 [M–100] ⁺	151.8–152.9, 80.7–81.7, 63.7–64.7, 57.7, 42.8
Cloprop	306.70	21.88	154.8–156.8 [M–150] ⁺	305.1–307.1, 177.9–178.9, 149.8, 122.8, 110.7, 90.7
2,4,5-T	361.64	32.35	135.83 [M–225] ⁺	59.1–361.1, 324.1–326.1, 206.8, 163.8, 149.8, 122.8, 106.7, 93.7, 90.7, 76.7
2,4-DB	355.25	37.72	191.8–192.9 [M–163] ⁺	149.8, 122.7–123.8

Table III. Recoveries, SD/(RSD) and F Values for Investigated Herbicides Spiked in Water Using LPE (n = 7), SPE (n = 4) and in Soil Samples (n = 3). The First Line Stands for Single Component Calibration Curves

Compound	Calc. µg/mL	Found µg/mL	Recovery %	SD* (RSD)	F
Water sample: LPE					
Baygon	20.90	19.99	95.7	1.57 (7.85)	7.33 (22)
Mixed component calibration curves		10.72	51.3	0.92 (8.61)	8.76 (22)
Cloprop	20.06	13.47	67.2	0.14 (1.07)	–
Mixed component calibration curves		55.26	275.5	0.79 (1.43)	–
2,4,5-T	25.54	15.48	60.6 (6.79)	1.05	1.72 (21)
Mixed component calibration curves		42.21	165.3 (6.92)	2.92	1.66 (21)
2,4-DB	24.91	20.44	82.1 (6.18)	1.26	1.60 (21)
Mixed component calibration curves		11.17	44.8 (6.09)	0.68	1.64 (21)
Water sample: SPE					
Baygon	20.90	6.13	29.3	0.20 (3.32)	1.27 (22)
Mixed component calibration curves		2.92	14.6	0.09 (3.14)	1.13 (22)
Cloprop	20.06	6.50	32.4 (1.81)	0.12	–
Mixed component calibration curves		18.57	92.6	0.69 (3.71)	–
2,4,5-T	25.54	0.63	2.5	0.04 (6.39)	1.96 (21)
Mixed component calibration curves		1.62	6.3	0.11 (6.52)	1.72 (21)
2,4-DB	24.91	14.05	56.4 (6.69)	0.94	1.36 (21)
Mixed component calibration curves		7.71	30.9	0.51 (6.61)	1.39 (21)
Soil sample					
Baygon	100.00	57.54	57.5	0.56 (0.98)	–
Mixed component calibration curves		27.57	27.6	0.29 (1.07)	–
Cloprop	100.00	53.15	53.2	3.43 (6.45)	–
Mixed component calibration curves		118.95	119.0	430 (2.92)	–
2,4,5-T	100.00	9.79	9.8	0.61 (6.16)	1.19 (23)
Mixed component calibration curves		25.32	25.3	1.59 (6.28)	1.17 (23)
2,4-DB	100.00	6.46	6.5	0.43 (6.65)	2.99 (24)
Mixed component calibration curves		3.68	3.7	0.24 (6.57)	2.77 (24)
* SD = standard deviation (based on mixed component calibration curves.)					

matographic Al₂O₃. The column was dried and eluted with 1000 mL of ethyl acetate, and ethylacetate was evaporated to dryness. The resulting residue was dissolved into 50 mL acetonitrile and derivatized by adding an equimolar amount of carbonyl dimidazole, stirring for 30 min, then adding 0.003 moles of methoxy aniline. The solution containing derivatized products was transferred into a volumetric flask and increased to 1000 mL using acetonitrile.

The resulting solution was subjected to GC–MS analysis under the previously given conditions. Recoveries were determined using the single and mixed component standard solution calibration curves.

Soil treatment

Soil samples (100–1000 g) were collected from Al-Ain [Abu-Dhabi, United Arab Emirates (UAE)], and they were sprayed with a mixture having 0.01 g each of the four investigated herbicides dissolved in acetonitrile. Irrigation was carried out once daily for 7 days. The soil was dried and extracted into 1.5 L of methanol over three extraction processes. The solvent was evaporated to dryness using a rotary evaporator. The resulting residue was dissolved into 50 mL acetonitrile, dried over anhydrous CaCl₂, filtered, and derivatized according to the derivatization procedure previously described. The resulting solutions, having the concentrations listed in Table III, were increased to 100 mL using acetonitrile. Solutions were subjected to GC–MS analysis under the previously given conditions. Recoveries for spiked soil samples were determined using the single and mixed component standard solution calibration curves.

Results and Discussion

Elemental analysis, IR, and NMR characterization of the amide derivatives

Phenoxy acid herbicides differ from each other in the length of the alkyl chain and the degree of substitution on the benzene ring. Their UV absorption spectra have λ_{max} at 230 and 280 nm. Therefore, derivatization processes were monitored

by following up the absorbance at 280 nm. The disappearance of the 280 nm band indicated the complete conversions of the acids into their amide derivatives. The formation of derivatized amides was also checked by C, H, N elemental analysis, shown in Table I. Good agreements between the calculated and found values confirmed the formation of amide derivatives. The IR spectra for derivatized phenoxy acids and their parent compounds are shown in Figure 2. A shift of the carbonyl band around $1720/\text{cm}^{-1}$ to a lower frequency and the appearance of a new sharp band around $3300/\text{cm}^{-1}$ indicated the formation of amide derivatives. A new signal appeared at a chemical shift of approximately 12.2 ppm in the $^1\text{H-NMR}$ spectra of derivatized phenoxy acids, relative to the parent compounds, giving additional evidence for derivatizing the acids into their amide derivatives.

Optimization of the derivatization reaction

The optimum derivatization reaction should result in a high derivatization yield, high reaction selectivity, the formation of a single product, the absence of byproducts, minimum sample work, no cleanup, and no analyte degradation. It also has to improve the detection limit and augment the selectivity by enhancing the hydrophobicity difference between analytes, resulting in high separation resolution. These conditions are fulfilled by the derivatization reaction selected for this investigation.

Carboxylic acids can be converted into amide, acylhydrazide, or ester derivatives. Because amides have a higher stability over acylhydrazides and esters, this approach was selected for this investigation. Figure 3 shows the formation mechanism of an amide derivative. The reaction was initiated by forming an ester bond between the deprotonated phenoxy acid and the carbonyl diimidazole. Subsequent rearrangements that led to the formation of an imidazole derivative are shown. The produced imidazole was then attacked by the derivatizing amine to form the amide. This coupling reaction also produced 1*H*-imidazole (25).

An optimum derivatization reaction was obtained in acetonitrile. Acetonitrile is a basic solvent that facilitates the deprotonation of the carboxylic acid group to initiate the reaction with carbonyl diimidazole (Figure 3). The reaction is completed by the nucleophilic attack of a methoxy aniline base on the imidazole derivative. Methoxy aniline showed better baseline resolution, a relatively short separation time, and enhanced the sensitivity for detecting the investigated phenoxy acids.

MS characterization of amide derivatives

Derivatized compounds were injected on the GC-MS either singly or in a mixture. Figure 4A shows the total ion current chromatogram for a sample containing the four derivatized compounds at a 50 ppm concentration using the SIM mode. Baygon, propionic acid, 2,4,5-T, and

butyric acid derivatives gave intense peaks at average retention times [$t_R \pm$ relative standard deviation (RSD)] of 14.74 ± 0.24 ($n = 23$), 21.89 ± 0.24 ($n = 22$), 32.35 ± 0.27 ($n = 21$), and 37.72 ± 0.37 ($n = 21$) min. An additional peak with the same mass spectra as the main product was observed for Baygon at

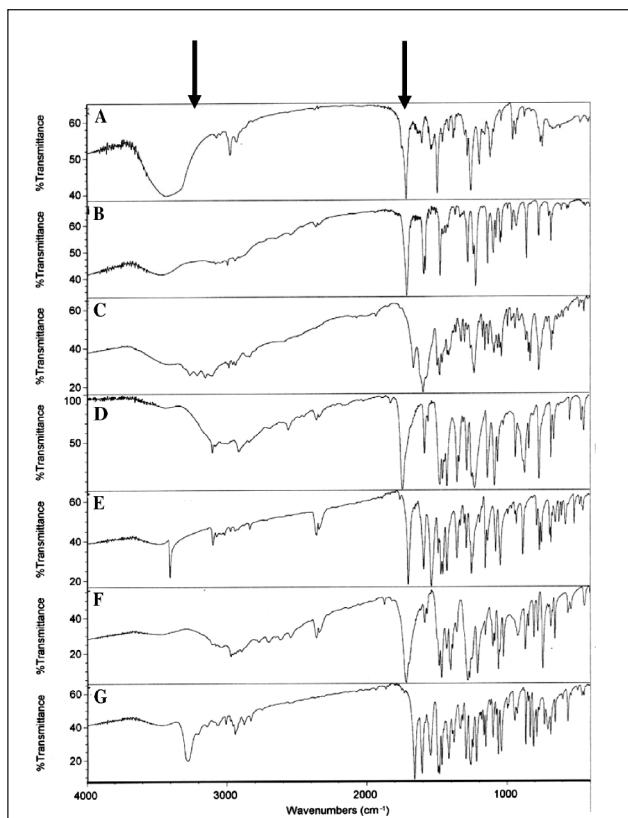


Figure 2. IR spectra of Baygon (A), Cloprop (B), 2-(3-chlorophenoxy)-propionic acid amide derivative (C), 2,4,5-T (D), 2,4,5-trichlorophenoxyacetic acid amide derivative (E), 2,4-D (F), and 4-(2,4-dichlorophenoxy)butyric acid amide derivative (G).

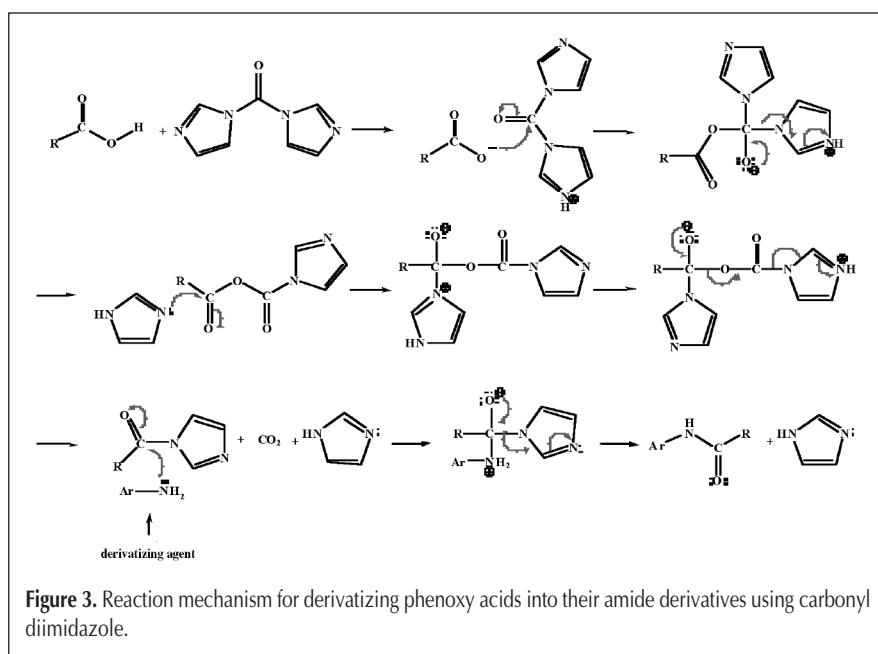


Figure 3. Reaction mechanism for derivatizing phenoxy acids into their amide derivatives using carbonyl diimidazole.

9.35 min. This peak was assumed to be attributed to an isomer present in the commercial standards.

Figures 5–8 show the MS fragmentation patterns for Baygon, Cloprop, 2,4,5-T, and 2,4-DB amide derivatives. In general, the mass spectra of the derivatized herbicides contained several intense cluster peaks that conserved the chlorine isotopic pattern, helping in their identification, even when complex matrix from soil or water samples were encountered. The most intense signals in the mass spectra of the amide compounds are given in Table II and shown in Figures 5–8.

The parent molecular ion signal for the amide derivatives was absent in the mass spectra of Baygon and 2,4-DB (Figures 5 and 8). The base peaks at m/z ($M-140$)⁺, ($M-150$)⁺, ($M-225$)⁺, and ($M-188$)⁺, corresponding to the loss of propyl-acetamide, *o*-methoxy-phenyl amide, trichlorophenyl methyl ester and dichlorophenol groups out of Baygon, Cloprop, 2,4,5-T, and 2,4-DB, respectively, are shown in Figures 5–8. A cluster of peaks corresponding to losses of smaller or higher masses were also observed. These ions could result from the loss of a phenolic methoxy group, methoxy aniline, methoxy phenyl acetamide, or propionamide.

Optimization of separation condition

Different temperature programming conditions were tested to attain the optimum separation conditions that have the highest intensity. The condition described in the Measurement parameters section gave the best resolution, peaks intensities, and shortest retention times. The SIM was shown to improve the sensitivity for derivatized compounds by 1 to 2 orders of magnitudes, relative to the full-scan monitoring mode (Figures 4A and 4B).

Linearity, precision, and limit of detection

The linearity of the GC–MS response for investigated amide derivatized herbicides was evaluated using the calibration curve method. Calibration curves based on standards having a single component or a mixture of the four compounds were constructed. Single component standards showed dynamic concentration ranges of 0.005–50, 0.05–50, 0.1–50, and 0.1–50 $\mu\text{g/mL}$ for Baygon (9A), Cloprop (9B), 2,4,5-T (9C), and 2,4-DB (9D) with

correlation coefficients greater than or equal to 0.97, respectively (Figure 9). The RSD for the retention times are less than or equal to 0.37% ($n \geq 20$). Detection limits of 1.0, 50.0, 100, and 1.0 ng/mL were obtained for Baygon, Cloprop, 2,4,5-T, and 2,4-DB, respectively, at a signal-to-noise of greater than or equal to 4 (Table IV). A set of calibration curves for stan-

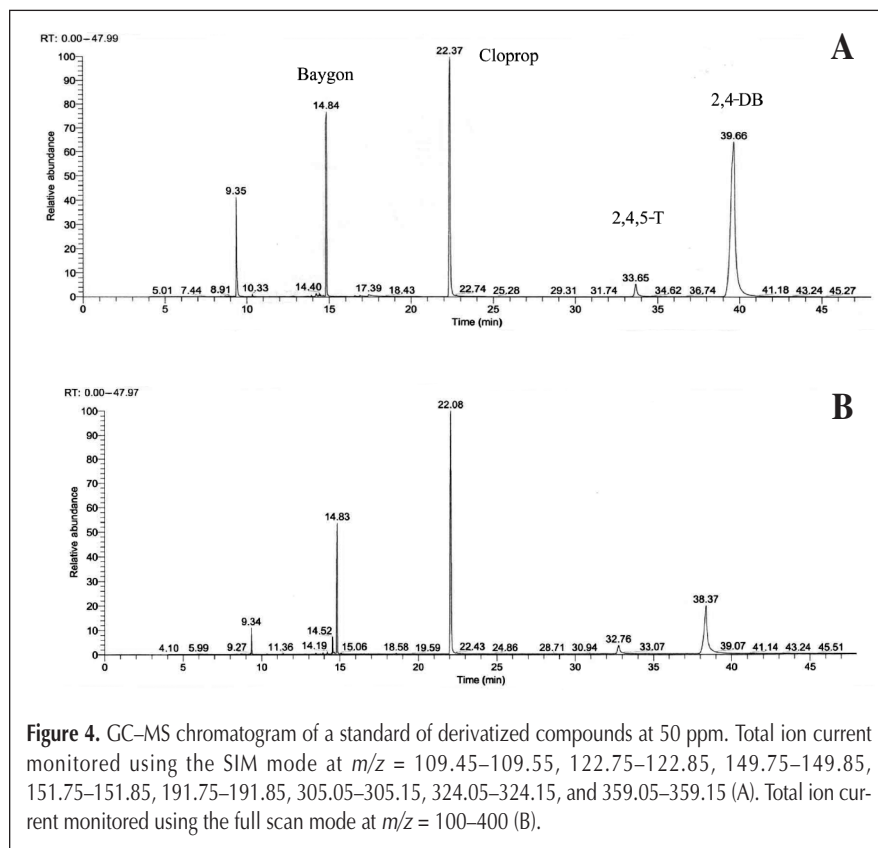


Figure 4. GC–MS chromatogram of a standard of derivatized compounds at 50 ppm. Total ion current monitored using the SIM mode at $m/z = 109.45\text{--}109.55$, $122.75\text{--}122.85$, $149.75\text{--}149.85$, $151.75\text{--}151.85$, $191.75\text{--}191.85$, $305.05\text{--}305.15$, $324.05\text{--}324.15$, and $359.05\text{--}359.15$ (A). Total ion current monitored using the full scan mode at $m/z = 100\text{--}400$ (B).

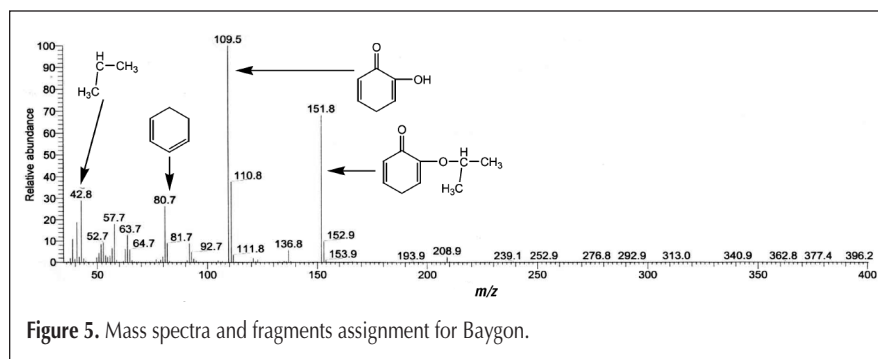


Figure 5. Mass spectra and fragments assignment for Baygon.

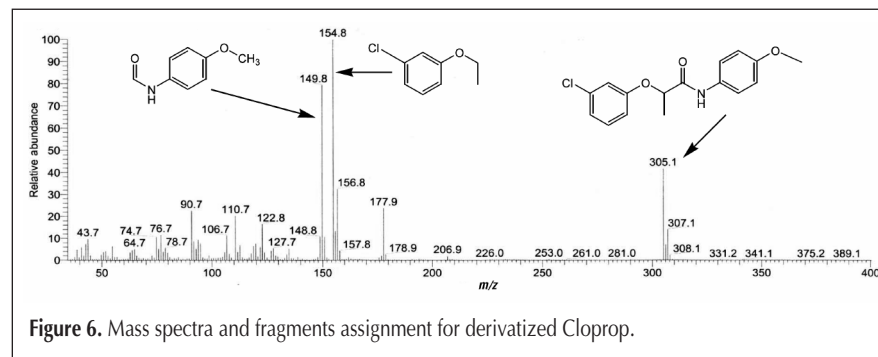


Figure 6. Mass spectra and fragments assignment for derivatized Cloprop.

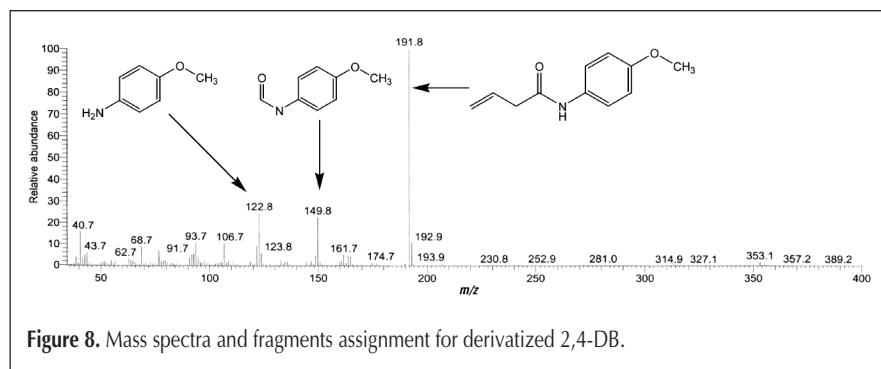
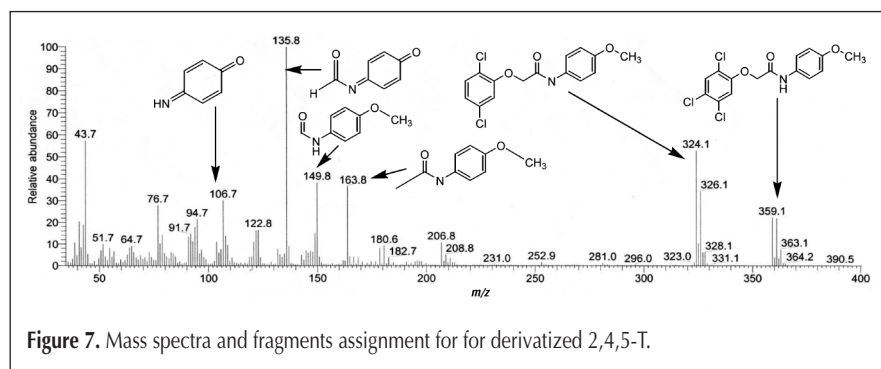
dards having a mixture of the four investigated compounds was constructed. The results are shown in Table IV.

Water samples: LPE and SPE

Spiked water samples were extracted using the LPE or SPE method previously described. Extracts were dried, dissolved in acetonitrile, and derivatized. Resultant solutions were assumed to have concentrations of 20–25 µg/mL in each her-

bicide. Produced samples were injected into the GC–MS system, and the data (peak area) were evaluated.

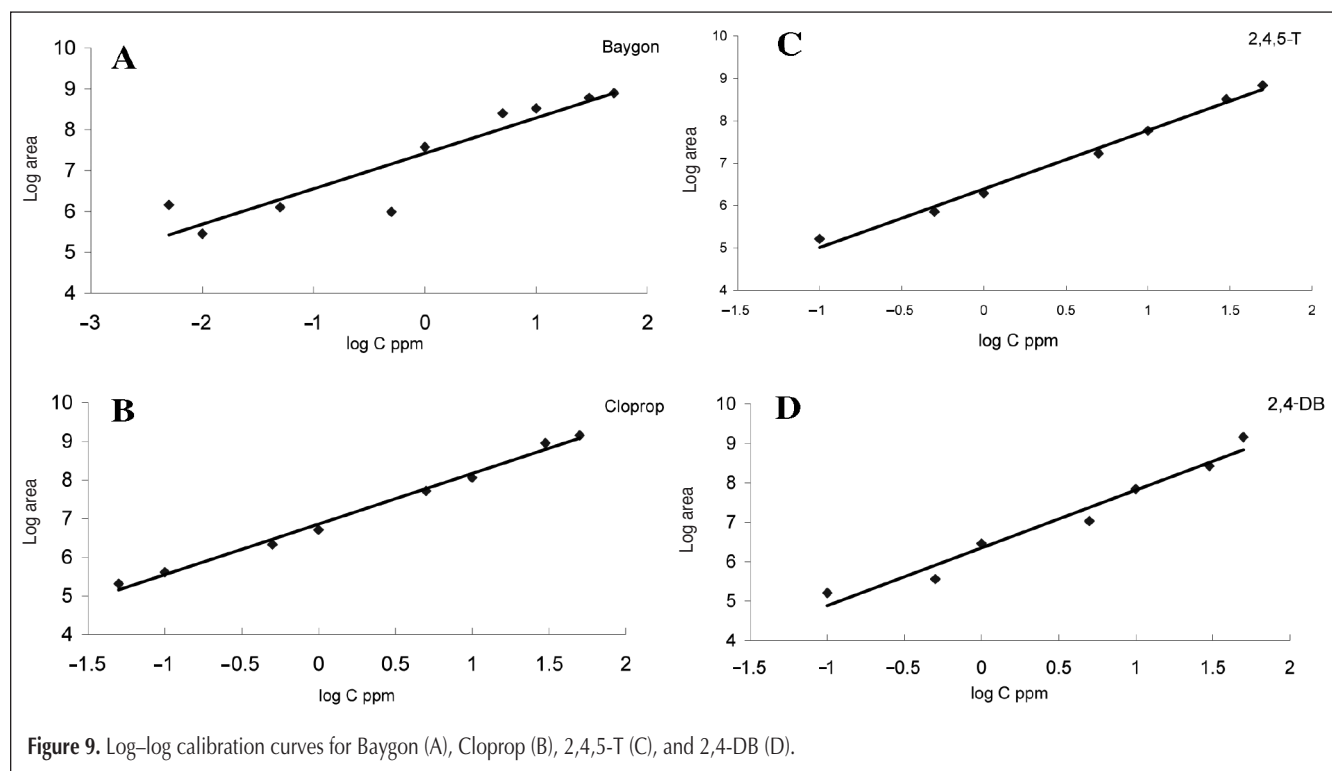
Recoveries between 60.6–95.7% and 2.5–56.4% were obtained using LPE and SPE based on single component calibration curves, respectively. Corresponding recoveries of 44.8–275.5% and 6.3–92.6% based on mixed component calibration curves were obtained. None of the four herbicides were detected in the non-spiked water samples (Table III).



Soil samples

The applicability of the developed method was tested by measuring soil samples treated with the four phenoxy acid herbicides. Soil extracts were derivatized and measured, and their recoveries were evaluated. Recoveries of 5.9–57.5% and 3.3–119.0%, based on single component and mixed component calibration curves, were obtained, respectively. None of the four herbicides were detected in the non-spiked soil samples (Table III).

Low recoveries were attributed to the low sorption affinity and phenoxy acid herbicides movement through the UAE soil. Cloprop, 2,4,5-T, and 2,4-DB showed low recoveries relative to Baygon in treated soil samples. This indicated that the extent of sorption/desorption was different from one herbicide to another. The difference can be rationalized based on the different hydrophobicity of individual herbicides. This



explanation was also supported by the low organic matter present in the soil. However, derivatized phenoxy acids from soil samples showed sensitive detection and eliminated interference from organic molecules that might exist in soil.

Recovery study and application of the method

Table III summarizes the average recovery of the four herbicides in fortified samples. Water LPE gave relatively better recovery than SPE from water and soil samples. Recoveries are generally found to decrease with an increasing number of chlorine atoms in the molecule. This may be attributed to the acidic strength as described by Notel and Kruger for phenoxy acidic herbicides (26) and Butte for phenols and fatty acids (27). LPE recoveries were satisfactory. The low sorption efficiency of Al_2O_3 and soil towards investigated herbicides was the cause of low recoveries for SPE and soil samples. Higher recoveries obtained using mixed component calibration curves might be attributed to the matrix effect.

Precision and detection limits

The average relative precisions ($n = 7$) for fortified water samples with 20.0–25.0 $\mu\text{g/mL}$ using LPE were 7.85%, 1.07%, 6.79%, and 6.18% for Baygon, Cloprop, 2,4,5-T, and 2,4-DB, respectively. Corresponding values using SPE ($n = 4$) were 3.32%, 1.81%, 6.39%, and 6.69%, respectively. Values based on mixed component calibration curves in ranges of 1.43–8.61% and 3.14–6.61% were obtained, respectively (Table III).

Three replicate soil samples fortified with 0.01 g in each pesticide gave average RSDs in the range 0.98–6.65% and 1.07–6.57% using single and mixed component calibration curves, respectively. Variation was mainly attributed to the weak sorption efficiency of Al_2O_3 and soil, as well as some minor matrix effects.

Applying the F-test at a 95% confidence level gave F values greater than 2.99, indicating insignificant differences in precisions between our developed method and previously published ones (21–24) (Table III). This clearly revealed that the

proposed methods were reliable, sensitive, simple, and practicable.

Conclusion

A successful, sensitive, selective, reliable, and simple analytical procedure for determining three phenoxy acid herbicides and one carbamate herbicide in water and soil samples was developed. Detection limits down to 1 ng/mL were obtained using the SIM-MS mode. Advantages over published methods include the commercial availability of the derivatization reagent, the rapidity of the reaction, and the quantitative derivatization yields. Isolated amides were found stable over several weeks. An MS of the amide derivatives showed rich fragmentation patterns facilitating their identification in sample extracts. Derivatization conditions were optimized with respect to the amounts of the analyte, amine, solvent, and derivatization reagent. Optimum derivatization yield was accomplished in acetonitrile. The reaction was completed in approximately 90 min. Interference from species existing in water and soil was substantially reduced.

The method simplifies sample preparation, avoids derivatization with hazardous reagents, and provides high precision. Silylation of phenoxy acid herbicides have not been recommended because the byproducts of the reaction led to a noisy GC baseline, which resulted in increasing the detection limits of the method (21). This method is better than that used in the literature (22,23) because of the simplicity and wide availability of the instrument; however, the latter methods showed higher sensitivity. This method is more sensitive compared with the method of Delgado et al. (24). It can be used as a screening tool to obtain information on sources, behavior, and the fate of the investigated herbicides in soil, as well as in surface and ground waters. F values less than or equal to 2.99 indicated insignificant differences in precisions between our developed method and previously published ones.

Table IV. Retention Times \pm its RSD ($n \sim 20$), Linear Logarithmic Regression Equations, Correlation Coefficients, Signal-to-Noise Ratios, and Detection Limits for Investigated Derivatized Herbicides Based on Single Component Calibration Curves. Values Between Parenthesis Stand for Mixed Component Calibration Curves. Signal-to-Noise at 1 ng/mL and 50 $\mu\text{g/mL}$ are Given

Compound	RT \pm RSD (min)	Regression equation	Corr. coeff. (R^2)	Signal-to-noise at (1–50 $\mu\text{g/mL}$)	Detection limit (ng/mL)
Baygon	14.74 \pm 0.24 (14.74 \pm 0.24)	Log A = 0.868 Log C + 7.422 (Log A = 0.796 Log C + 7.803)	0.99 (0.96)	10–15012 (10–12763)	1.00 (1.00)
Cloprop	21.89 \pm 0.17 (21.88 \pm 0.19)	Log A = 1.309 Log C + 6.860 (Log A = 0.802 Log C + 7.437)	0.99 (0.92)	4–427 (12–801)	50.00 (50.00)
2,4,5-T	32.35 \pm 0.27 (32.35 \pm 0.28)	Log A = 1.383 Log C + 6.393 (Log A = 1.357 Log C + 5.860)	0.99 (0.98)	4–493 (4–288)	100.00 (100.00)
2,4-DB	37.72 \pm 0.37 (37.73 \pm 0.36)	Log A = 1.464 Log C + 6.350 (Log A = 1.482 Log C + 6.697)	0.97 (0.99)	10–58504 (17–2109)	1.00 (1.00)

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References

1. E. Macutkiewicz, M. Rompa, and B. Zygmunt. Sample preparation and chromatographic analysis of acidic herbicides in soils and sediments. *Crit. Rev. Anal. Chem.* **33(1)**: 1–17 (2003).
2. R. Soniassy, P. Sandra, and C. Schlett. *Water Analysis: Organic Micropollutants*. Hewlett-Packard, Germany, 1994.
3. L.H. Keith (Ed.). *Environmental Endocrine Disruptors*. Wiley, New York, NY, 1997.
4. E.M. Lores, T.R. Edgerton, and R.F. Moseman. Method for the confirmation of chlorophenols in human urine by LC with an electrochemical detector. *J. Chromatogr. Sci.* **19(9)**: 466–69 (1981).
5. C.W. Thorstensen, O. Lode, and A.L. Christiansen. Development of a solid-phase extraction method for phenoxy acids and bentazone in water and comparison to a liquid–liquid extraction method. *J. Agric. Food Chem.* **48(12)**: 5829–33 (2000).
6. T.S. Thompson and L. Morphy. Rapid extraction of chlorinated phenoxyacid herbicides from drinking water using. *J. Chromatogr. Sci.* **33**: 393–98 (1995).
7. S. Butz and H.J. Stan. Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary gas chromatography of their alkyl esters formed by rapid derivatization using various chloroformates. *J. Chromatogr. A* **643**: 227–38 (1993).
8. T.D. Bucheli, F.C. Gruebler, S.R. Müller, and R.P. Schwarzenbach. Simultaneous determination of neutral and acidic pesticides in natural waters at the low nanogram per liter level. *Anal. Chem.* **69(8)**: 1569–76 (1997).
9. W.H. Ding, C.H. Liu, and S.P. Yeh. Analysis of chlorophenoxy acid herbicides in water by large-volume on-line derivatization and gas chromatography–mass spectrometry. *J. Chromatogr. A* **896**: 111–16 (2000).
10. T. Henriksen, B. Svensmark, B. Lindhardt, and R.K. Juhler. Analysis of acidic pesticides using in situ derivatization with alkylchloroformate and solid-phase microextraction (SPME) for GC–MS. *Chemosphere* **44**: 1531–39 (2001).
11. T. Nilsson, B. Baglio, I. Galdo-Miguez, J.O. Madsen, and S. Facchetti. Derivatization and solid-phase microextraction followed by gas chromatography–mass spectrometry for the analysis of phenoxy acid herbicides in aqueous samples. *J. Chromatogr. A* **826**: 211–16 (1998).
12. M.R. Lee, R.J. Lee, Y.W. Lin, C.M. Chen, and B.H. Hwang. Gas-phase postderivatization following solid-phase microextraction for determining acidic herbicides in water. *Anal. Chem.* **70(9)**: 1963–68 (1998).
13. H.D. Meiring, G. Engelsman, and A.P.J.M. de Jong. Determination of polar pesticides by phase-transfer catalysed derivatization and negative-ion chemical ionization gas chromatography–mass spectrometry. *J. Chromatogr. A* **644**: 357–65 (1993).
14. L.B. Olsen. Intra-injector formation of methyl esters from phenoxy acid pesticides. *J. Chromatogr. A* **598**: 309–12 (1992).
15. H.G.J. Mol, S. Sunarto, and O.M. Steijger. Determination of endocrine disruptors in water after derivatization with *N*-methyl-*N*-(tert.-butyldimethyltrifluoroacetamide) using gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **879**: 97–112 (2000).
16. I. Rodryguez, J.B. Quintana, J. Carpinteiro, A.M. Carro, R.A. Lorenzo, and R. Cela. Determination of acidic drugs in sewage water by gas chromatography mass spectrometry as tert-butyldimethylsilyl derivatives. *J. Chromatogr. A* **985**: 265–74 (2003).
17. T. Heberer and H.J. Stan. Detection of more than 50 substituted phenols as their *t*-butyldimethylsilyl derivatives using gas chromatography–mass spectrometry. *Anal. Chim. Acta.* **341**: 21–34 (1997).
18. M.J. Bertrand, A.W. Ahmed, B. Sarrasin, and V.N. Mallet. Gas chromatographic and mass spectrometric determination of chlorophenoxy acids and related herbicides as their (cyano ethyl)dimethylsilyl derivatives. *Anal. Chem.* **59(9)**: 1302–06 (1987).
19. X. Lou, D.J. Miller, and S.B. Hawthorne. Static subcritical water extraction combined with anion exchange disk sorption for determining chlorinated acid herbicides in soil. *Anal. Chem.* **72(3)**: 481–88 (2000).
20. I.R. Pereiro, R.G. Irimia, E.R. Cano, and R.C. Torrijos. Optimisation of a gas chromatographic mass spectrometric method for the determination of phenoxy acid herbicides in water samples as silyl derivatives. *Anal. Chim. Acta.* **524**: 249–56 (2004).
21. D.J. Munch and A.M. Pawlecki-Vonderheide. Application of the base promoted esterification procedure to the chlorophenoxy acid herbicides of U.S. EPA Method 515.3. *J. Chromatogr. Sci.* **36(6)**: 279–83 (1998).
22. J.D. Ragsdale and M. Conoley. Thermo Finnigan Application Note no 9168: 1. Thermo Finnigan, Hertfordshire, UK, 2001.
23. J.D. Ragsdale and M. Conoley, Thermo Finnigan Application Note no 9170: 1. Thermo Finnigan, Hertfordshire, UK, 2001.
24. M.J.S. Delgado, E.C. Corral and L.M. Poldiez. Determination of herbicides in soil samples by gas chromatography: optimization by the simplex method. *Talanta* **53**: 367–77 (2000).
25. R. Bruckner. *Advanced Organic Chemistry Reaction Mechanisms*. Harcourt Science and Technology academic Press, Massachusetts, 2002.
26. J. Nolte and R. Kruger. Direct methylation at the surface of Carboxypack B: Part II: Determination of phenoxyalkanoic acid herbicides. *Fresenius J. Anal. Chem.* **365(7)**: 610–14 (1999).
27. W. Butte, J. Eilers, and M. Kirsch. Trialkylsulphonium and trialkylselenonium hydroxides for the pyrolytic alkylation of acidic compounds. *Anal. Lett.* **15**: 841–50 (1982).

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