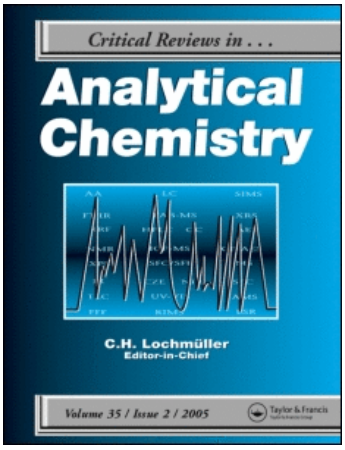

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Sample Preparation and Chromatographic Analysis of Acidic Herbicides in Soils and Sediments

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ABSTRACT: This article reviews sample preparation of soil and sediment samples for the chromatographic determination of acidic herbicides of phenolic and carboxylic acid classes. The common methods of acidic herbicides enrichment and isolation from soils and sediments were discussed, mainly supercritical fluid extraction, pressurized solvent extraction, subcritical water extraction, microwave-assisted extraction, and also traditional extraction with organic solvents and aqueous basic solutions. Derivatization to make herbicides analyzable by means of gas chromatography and/or to improve detectability by GC and LC — and also extraction efficiency were described.

KEY WORDS: acidic herbicides, GC analysis, LC analysis, derivatization, soils and sediments.

I. INTRODUCTION

Soil is one of the most valuable properties for men; biological production takes place there. Soil plays an important role in the hydrologic cycle, affects water quality and determines the behavior of ecological land systems.

Soil consists of mineral matter (45 to 50%), and water (20 to 25%) and air (25 to 30%), which fill inter- and intraparticle spaces. Soil is a source of food for vegetation and animal and vegetable microorganisms (0.1 to 7%).[1,2,3] The classification of mineral components according to particle size gives the following soil categories: sand (0.02 to 2 mm), silt (0.002 to 0.02 mm), and clay (less than 0.002 mm). Organic matter can be divided into humic and nonhumic. The latter constitutes only 10 to 15% soil organic matter.[3,4] Specific surface area, an important characteristic of soil, ranges from 10 to 40 m^2/g for sandy loam to 150 to 250 m^2/g for clay soil. Clay is a soil component with the highest sorption properties. Soil water content changes with depth; the upper layer generally has smaller water content. The gaseous phase in soil is the modified air with the increased content of carbon dioxide (up to 1%) and the decreased content of oxygen. Soil air contains detectable amounts of other metabolic products, such as ammonia, methane and hydrogen sulfide. Soils differ in organic matter content and some other characteristics.[1]

The evaluation of a natural level of trace elements is difficult because a majority of soil is affected by men activity. It has been estimated that approximately half of soil resources have been degraded due to demographic expansion and industrialization. The process is estimated to continue at a rate of 0.5% per year.[2]

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An important group of anthropogenic pollutants of soil are herbicides, which can also be detected in water and some microorganisms. Herbicides presence in the environment results from their wide use on a global scale (annually approximately 2.5 mln ton).[5] Phenoxy acids, which have been used as herbicides since the 1940s to damage wide-leaved weeds and grass are simultaneously the benefit and threat to the environment. These synthetic growth regulators accumulate in growing parts of roots and stems. At low concentrations they (e.g. 2,4-D) can increase crops, while at high concentrations act as growth inhibitors. They have damaging effects due to high physiological activity and structure diversity.

Acidic herbicides (phenoxy acids, phenolic compounds) are widely used in agriculture and forestry; they are also applied to clean up sport areas, railways, highways, cemeteries, and also to control seaweed and other vegetation in marshy areas, water bodies, and drainage ditches.[6] In Europe a majority of the herbicides used are acidic herbicides;[7,8] in Sweden 65% of herbicides applied are phenoxy acids.

Tens of compounds from different chemical classes are used as acidic herbicides. They are derivatives of: phenol (dinoseb, dinoterb, pentachlorophenol); benzoic acid (sodium and ammonium salts of 3,6-dichloro-2-methoxybenzoic acid–dicamba, sodium, and ammonium salts of 3-amino-2,5-dichlorobenzoic acid–chloramben, etc.); acetic acid ((2,4-dichlorophenoxy)acetic acid (2,4-D), (4-chloro-2-methylphenoxy)acetic acid (MCPA)); propanoic acid(dichlorprop, 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP), etc.); butanoic acid4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB); and other acids (e.g., potassium or triisopropylamine salt of 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid–pikloram, triethylammonium 3,5,6-trichloro-2-pyridyloxyacetate-triclopyr-TEA). Characteristics and applications of acidic herbicides are described by Hornsby, Wauchope, and Herner.[9] Systematic names and structural formulas of selected herbicides are given in Table 1.

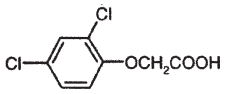
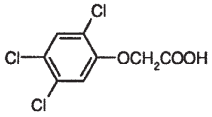
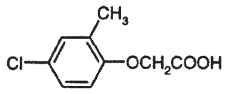
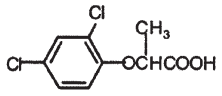
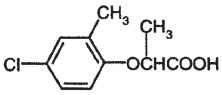
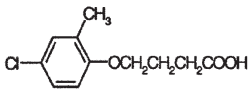
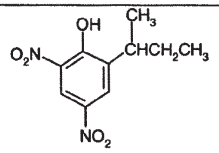
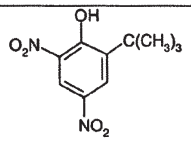
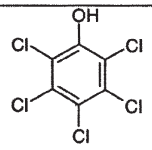
In commercial formulations acidic herbicides are present as metal or alkylammonium salts, alkyl esters and free acids.[13]

Due to relatively good solubility in water, acidic herbicides easily enter different water bodies and other environmental systems. If their degradation rate is not large enough, they can have harmful effects on soil microflora, can enter food, and then the human body.

Acidic herbicides are toxic for many living organisms, although none belongs to a group of very toxic pollutants. Some are mutagenic, teratogenic, and carcenogenic; some have detrimental effects on mammals causing pyrexia, nausea, hypotonia, coma, metabolic acidosis, convulsions, cytoskeletal perturbation, and renal damage. Toxicity of phenoxy acids changes from compound to compound; lethal dose (LD_{50}) ranges from 300 to 3000 mg/kg body weight. For example, 2,4-D is teratogenic and carcenogenic. Once present in the stomach, 2,4-D easily enters the blood and reaches a high concentration; however, it is nearly completely removed from a body within 24 to 48 h. Phenolic herbicides, for example, dinitrophenol damage liver parenchyma, kidneys, nerve muscle, and mucous membrane and cause skin and mucosa irritation.[6,14,15] Therefore, acidic herbicides should be monitored in environmental samples even when present at low concentrations.

Matrix complexity and the fact that acidic herbicides should be monitored at low concentrations make that analytical methods of high sensitivity, selectivity, and resolution power must be applied for their determination in soil, sediments, water, and other environmental samples.[16-18] It is not surprising then that chromatographic methods, most often gas chromatography (GC) and liquid chromatography (LC), are applied. However, environmental samples must generally be

TABLE 1
Chemical Formulas of Selected Acidic Herbicides (Phenoxyalkanocarboxylic Acids and Phenol Derivatives)[6,9-12]

	Herbicide	Structure
Derivatives of acetic acid	**2,4-D** (2,4-dichlorophenoxy)acetic acid $C_8H_6Cl_2O_3$	
	2,4,5-T (2,4,5-trichlorophenoxy)acetic acid $C_8H_5Cl_3O_3$	
	MCPA (4-chloro-2-methylphenoxy)acetic acid $C_9H_9ClO_3$	
Derivatives of propanoic acid	**Dichlorprop** **2,4-DP** 2-(2,4-dichlorophenoxy)propanoic acid $C_9H_8Cl_2O_3$	
	MCPP 2-(4-chloro-2-methylphenoxy)propanoic acid $C_{10}H_{11}ClO$	
Derivatives of butanoic acid	**MCPB** 4-(4-chloro-2-methylphenoxy)butanoic acid $C_{11}H_{13}ClO_3$	
Derivatives of phenol	**Dinoseb** 2-(1-methylpropyl)-4,6-dinitrophenol $C_{10}H_{12}N_2O_5$	
	Dinoterb 2-(1,1-dimethylethyl)-4,6dinitrophenol $C_{10}H_{12}N_2O_5$	
	PCP Pentachlorophenol C_6HCl_5O	

subjected to deep treatment before chromatography is applied. Soil samples are especially difficult to analyze and their preparation for chromatographic analysis generally consists of a few successive steps. The preparation is based on isolation of acidic herbicides of interest, removal of interfering substances, and transfer of analytes to an appropriate solvent.[19] Soil components most interfering with herbicide determination include:

- high molecular substances (fats, waxes) of molecular masses in the range of 600 to 1500. These are a mixture of long-chain alcohols, acids and esters; all these compounds are able to form hydrogen bonds and have low volatility;
- elemental sulfur (S_8);
- substances of a molecular mass close to that of herbicide analytes; these accompanying substances can have physicochemical properties similar to or different from analytes.

In the case of gas chromatography, which is the most often used for final analysis, carboxylic acid herbicides must and phenolic herbicides should be converted to less polar and more volatile derivatives.[20] Acidic herbicides are also often derivatized for liquid chromatographic analysis to improve detection limits.

In the case of both gas and liquid chromatography, sample preparation is generally a complex procedure requiring a lot of care. This problem should be comprehensively discussed.

II. SAMPLE PREPARATION FOR GAS CHROMATOGRAPHY

The preparation of soil and sediment samples for GC analysis generally includes the isolation of acidic herbicides, clean-up of extracts, analytes enrichment, often accompanied by solvent exchange, and derivatization at a certain step of the procedure.

The interaction of herbicides with a soil matrix is stronger than with water or food, and some fraction of them can be strongly bonded to soil matrix. Extraction procedures should be capable of liberating a bonded fraction also. Usually the extraction of a matrix bonded fraction requires a prolonged contact time of a sample with an extraction fluid, and contact enhancement by shaking, sonication, and pressure or temperature increase. Soxhlet extraction has proven quite efficient and has been applied successfully for over 30 years. It is time consuming, but the most exhaustive and therefore often applied as a reference method for soil extraction. Other traditional extraction methods are generally also time consuming and labor intensive, and therefore more automated procedures are sought for, especially when multiple operations of extraction, enrichment, and clean-up are necessary.

In recent years quite a few new sample preparation methods have been developed, including microwave assisted extraction (MAE), sonication, supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE).[21-23] In all these techniques smaller quantities of solvents are used, extraction time is shorter, and efficiency often increased.

Quite recently applicability of subcritical water for the extraction of polar and nonpolar trace organics from solid environmental samples has been shown. Lou et al.[24] applied such an approach to extract many acidic herbicides (pentachlorophenol, dinoseb, 3,5-dichlorobenzoic acid, dicamba, 2,4-DP, 2,4-D, 2,4,5-TP, 2,4,5-T, 2,4-DB) from soil. An aqueous extractant has remarkable advantages — it is cheap, environmentally friendly, and its polarity can be modified simply by a temperature change. Many acidic herbicides show quite good solubility in water, and subcritical water was assumed

to be an efficient extractant for these compounds in soil matrices. An additional advantage can be that, under certain conditions, herbicides in the form of esters can undergo hydrolysis in water. The hydrolysis, when necessary, can be carried out simultaneously with extraction. Moreover, many extraction procedures for aqueous samples can be directly applied to soil extracts. The method does not consume organic solvent at a step of soil extraction. The apparatus is simple and quite cheap.

Lou et al.[24] combined subcritical water extraction (SbWE) with strong anion exchange (SAX). In their experiments a soil sample and a SAX disk were placed in an extraction cell to which water was added. The cell was heated for some time and then cooled. In the combined SbWE/SAX system herbicides were extracted from soil to subcritical water and then to the SAX disk. They were eluted from the disk, derivatized, and analyzed by means of GC equipped with ECD or GC coupled with MS.

Lou et al.[24] tested five derivatizing reagentsC_2H_5I, CH_3I, BF_3-CH_3OH (10%), BSTFA (*N,O-bis*(trimethylsilyl)trifluoroacetamide) and TMCS (trimethylchlorosilane)-BSTFA (1%). BSTFA proved the most preferred, because this derivatizing agent and reaction byproducts are volatile and can cause negligible chromatographic interferences. Moreover, BSTFA can be used without any additional organic solvents, and a mixture after derivatization can be directly injected to GC with no observable negative effects on the system. With BSTFA quantitative derivatization can be achieved directly on the extraction disk, which can be placed in an autosampler vial for analytes derivatization and injection into GC.

The method was tested on pure sea sand, sandy soil (0.3% organic matter), agricultural soil (2% organic matter), and garden soil (ca. 12% organic matter). For all the samples studied extraction yield increased with temperature from 75°C to 100°C, and then decreased, probably due to faster herbicide degradation at higher temperatures.

For SAX trapping, the authors[24] hydrolyzed acidic herbicides in a form of esters and ethers to the corresponding acids or phenols. The hydrolysis was not effective in pure water but in the presence of soil hydrolysis rate increased, as they showed for esters of chlorophenoxy acids (2,4-DP, 2,4-D, 2,4,5-TP, 2,4,5-T, 2,4-DB). This agrees with the proven observation that in agricultural land esters undergo hydrolysis relatively fast.

Opposite from a conventional method, method 8150 recommended by the US Environmental Protection Agency (EPA), consists of many steps (extraction, hydrolysis, diazomethane derivatization), a method proposed by Lou et al.[24] based on SbWE/SAX is relatively fast and consumes only small amounts of organic solvents. The total analysis time, including extraction, derivatization, and GC separation, is shorter than 2 h, and the apparatus is quite simple.

Chiang et al.[25] developed a rapid extraction-methylation procedure for gas chromatographic analysis of four chlorinated phenoxy acetic acids (2,3- and 2,4-D; 2,4,5-T; MCPP) in soil samples. The four acidic herbicides were converted *in situ* to methyl esters during extraction. Esterification is one of the reactions used most frequently to derivatize acid herbicides, such as chlorophenoxyacetic acids, which have high melting points (> 120°C), high polarity, and a tendency to form dimers. The formation of methyl esters is particularly preferred, because they can easily be prepared and have reasonably short GC retention times.

As derivatizing reagents Chiang et al.[25] used: tetramethylammonium hydroxide (TMAH), benzyltrimethylammonium hydroxide (BTMAH) and its methoxide, bromide, and chloride analogues (BTMAM, BTMAB, BTMAC) and benzyltriethylammonium chloride (BTEAC) (all in the form of methanol solutions). TMAH appeared to

be the least satisfactory, while very high recoveries were obtained with BTMAB, BTMAC, and BTEAC, which was the most efficient.

In the studies a lunette soil (sandy loam) was used. Its composition was 4.07% organic matter, 10.60% clay, 33.26% silt, and 56.14% sand and pH 6.15. The soil was passed through a 100-mesh sieve and dried at 105°C for 24 h. Then it was equilibrated with water and 1% sulfuric acid. Methanol solutions of the free acidic herbicides were added making concentrations in the soil of 2 and 0.4 μg/g, and then equilibrated. BTMA-type reagents in methanol were used for *in situ* derivatization. The filtrate was collected and the liquid phase containing methyl esters concentrated by evaporation at 35°C under a gentle stream of nitrogen. The methyl esters of MCPP, 2,3-D and 2,4-D are volatile and easily lost with harsher treatment. The methyl esters were transferred to hexane, concentrated, cleaned up on a Florisil, column and analyzed by GC. The recoveries and precisions for all four herbicides were quite good — 96.6 ± 2.1% for 2,3-D; 96.4 ± 2.8% for 2,4-D; 96.6 ± 2.3% for 2,4,5-T; 94.6 ± 3.0% for MCPP (at a concentration level of 2.0 μg/g).

Tsukioka and Murakami[26] proposed a method for determination of eight typical herbicides in soil based on extraction with aqueous $Ca(OH)_2$ solution and final GC-MS analysis. The herbicides studied were 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP), 3,6-dichloro-2-methoxybenzoic acid (dicamba), 2-methyl-4-chlorophenoxyacetic acid (MCP), 2,3,6-trichlorobenzoic acid (TBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridyloxyacetic acid (triclopyr), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), α-(2-methyl-4-chlorophenoxy)butyric acid (MCPB). Samples of polluted soil were treated with calcium hydroxide solution and sonicated. After centrifuging, the extract was passed through a glass wool filter. Extraction was repeated with distilled water. The combined filtrates acidified to pH = 1 were extracted with CH_2Cl_2. After exchanging the solvent for acetone, herbicides were derivatized with pentafluorobenzyl bromide (PFBB) under basic conditions (K_2CO_3). A reaction mixture was evaporated to dryness, the residue dissolved in hexane and the concentrate analyzed by means of GC-MS (SIM mode). Recoveries were above 89% (except TBA — 77%) and precision expressed as a relative standard deviation less than 5%.

Ngan and Ikesaki[11] extracted nine acidic herbicides (seven phenoxy acids, one phenolic herbicide, and chlorobenzoic acid) with ethyl ether and then estrified with diazomethane according to the procedure they developed. Diazomethane was produced from Diazald when needed. This modified methylation procedure has obvious advantages: diazomethane distillation is not needed, which eliminates explosion danger; it is fast and simple; Diazald is a safe compound and more stable than earlier applied 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) and gives higher amounts of diazomethane. Esters were sorbed in cartridges packed with magnesium silicate (Florosil), eluted with CH_2Cl_2, solvent exchanged for *n*-hexane and concentrate analyzed by means of GC-ECD. Average recoveries were higher than 86%; after clean-up on a Florosil column higher than 89%.

A relatively new and fast method of organic pollutants isolation from soil samples is supercritical fluid extraction (SFE). In the case of acidic herbicides, it was first applied by Rochette et al.[27] for determination of 2,4-D in soil (final analysis by GC-ECD). In the method, CO_2 is used most often, less often N_2O, and only rarely other substances in supercritical state. CO_2 is relatively cheap, easily available in the ultra-pure state, what is very important in trace organic analysis, and has relatively low critical parameters — it was applied in the above experiments. However, CO_2 is nonpolar and has poor extraction strength for polar acidic herbicides.

Extraction strength with respect to polar analytes is generally increased with the addition of organic modifiers (methanol, acetonitrile, ethanol) that do not increase critical parameters drastically. Extraction yields can also be increased by addition of appropriate reagents that react either with matrix or rather with an analyte of interest.

They compared four derivatization reactions run under SFE conditions: silylation, methylation, ion paring, and ion exchange. In absence of soil, silylation of 2,4-D was effective (ca. 91% recovery), in the presence of soil the recovery was three times lower. They also[27] presumed that the silylation reaction can be disturbed by water present in soil (0.4%). An additional disadvantage is that silyl derivatives of 2,4-D are not commercially available, which makes standard preparation difficult.

Conversion of 2,4-D in soil to methyl esters with the use of a mixture of boron trifluoride and methanol under SFE conditions was studied not only by Rochette et al.[27] but also by Hawthorne.[28] Both research groups obtained identical results though the former state that, after extraction, BF_3 should be removed and methyl ester of 2,4-D transferred to nonpolar solvent (e.g., benzene) to prevent a chromatographic column from damage. The presence of BF_3 in samples injected to GC results in peaks broadening and damaging PEEK made sealings. Therefore, BF_3 is not recommended for *in situ* derivatization despite that recoveries can be quite high (ca. 90% for 2,4-D).

For ion pairing Rochette et al.[27] used *m*-trifluoromethylphenyltrimethylammonium hydroxide (TFTMPA), which gives a salt well soluble in supercritical fluid (SF). In a GC injection port the ion pair thermally decomposes to 2,4-D methyl ester. The application of TFTMPA or other tetraalkylammonium hydroxide is a good alternative to BF_3/methanol derivatization, because extracts do not need additional treatment. However, recoveries were lower than for 2,4-D methyl ester standards.

For the selection of the best reagent for ion exchange, they[27] conducted many experiments. The best reagent mixture was found to be $CaCl_2$ solution in methanol giving a recovery of 87%. Earlier Cheng[29] showed that this solution was the best reagent for ion pairing in liquid-liquid extraction (LLE). The studies by Rochette et al.[27] were aimed at selecting the most effective method of sample pretreatment before SFE. Average recoveries of 2,4-D (six independent measurements) for different derivatization reactions were as follows: 31% (RSD 9%) for silylation, 90% for a mixture of BF_3 and methanol, 14 to 19% for ion pairing, and 86 to 87% for ion exchange.

Also Lopez-Avila et al.[30] used derivatization-SFE for the isolation of chlorophenoxyalkanoic acid herbicides from soil samples. Supercritical CO_2 was used as an extractant and tetrabutylammonium hydroxide (TBA) and methyl iodide (MI) as a derivatizing mixture. Attempts to apply other reagents such as TMPA, benzyltrimethylammonium chloride (BTMAC), and benzyltriethylammonium chloride (BTEAC) were also made. These reagents were less effective than a mixture of TBA and MI; TBA ionises compounds and MI is an alkylating agent. They studied such herbicides as dicamba, MCPP, MCPA, 2,4-D, 2,4,5-T, MCPB, and 2,4-DB. The matrices were a topsoil (58% sand, 22% silt, 20% clay, pH 7.5, moisture content 2.6%; organic carbon content 0.1%), a clay soil (34% sand, 35% silt, 31% clay; pH 7.4; moisture content 10.6%; organic carbon content 1.8%), and a sand whose composition was not known.

Soils and sand were spiked with a concentrated stock solution of chlorophenoxy acid herbicides in methanol. After solvent evaporation, the spiked sample was derivatized in the extraction vessel with a selected derivatizing

reagent (e.g., TMPA, BTMAC, BTEAC, or TBA/MI).

All extractions were carried out at 40 MPa and two temperatures of 80 and 100°C, dynamic extraction was preceded by static extraction to allow longer contact of TBA and MI with the matrix. The extracted analytes were collected in methanol.

For optimization of TBA/MI excess, various amounts of TBA and MI were added to standards of dicamba and 2,4-D, and the mixtures were immediately injected into a GC-MS system.

To determine the reproducibility of methylation reaction, standards of seven chlorophenoxy acids in methanol with TBA/MI were injected into a GC-MS system.

Lopez-Avila et al.[30] also determined recoveries in a SFE-TBA/MI procedure. Tests were done for three different matrices (sand, clay soil, and topsoil) spiked at two concentrations (50 and 250 μg/g) with chlorophenoxy acids. Extraction was performed immediately after spiking.

Acidic herbicides extraction by means of SF CO_2 modified with 5 and 10% methanol was also investigated. Extraction was conducted at 40 MPa and 80°C first in the static mode and then in the dynamic mode. Because the acids need to be derivatized prior to GC-MS analysis, TBA and MI were added to a collection solvent.

After injection of the extract into the GC-MS system, the acids are converted to their corresponding methyl esters. The minimum injection temperature at which the methylation reaction occurs is about 100°C as Lopez-Avila et al. determined in separate experiments.

Under optimal excess of TBA and MI, the average derivatization yields were 84% (2.2% RSD) for dicamba and 100% (1.3% RSD) for 2,4-D.

Studies on the methylation reaction of seven chlorophenoxy acids showed that the derivatization yields ranged from 46.4 to 93.6%, with two compounds exhibiting average yields lower than 70% and reproducibility expressed as RSD better than 8.6% (one case — 16.6%).

The recoveries in the whole isolation process (SFE-TBA/MI) for three soils ranged from 57.4 to 141% (average 95.5%).

The authors also studied the effect of organic modifiers on chlorophenoxy acids recovery.

The SFE procedure with TBA/MI derivatization is applicable to the qualitative determination of chlorophenoxy acid herbicides in soil samples at mg/g levels. Because the extraction is performed with supercritical carbon dioxide (a nontoxic, nonflammable, nonpolluting, and relatively inexpensive material) and is fast (typically 30 min for a 2 to 5 g sample), the method proposed by Lopez-Avila et al. is an attractive alternative to conventional Soxhlet extraction procedures specified in EPA SW-846 Methods 8150B and 8151. Furthermore, TBA and MI are less toxic than the reagents used in the diazomethane procedure recommended by EPA (Method 8150B).

Hawthorne et al.[31] investigated isolation of 2,4-D and dicamba from soil and sediments in a procedure based on SFE — *in situ* chemical derivatization using such reagents as trimethylphenylammonium hydroxide (TMAH) and boron trifluoride in methanol.

Chemical derivatization-SFE was performed in a two-step procedure consisting of analytes derivatization under static SFE conditions, and recovery of the derivatized analytes using dynamic SFE. The procedure was conducted with CO_2 at 40 MPa and 80°C. Extracted analytes were collected in methanol or methylene chloride. All extracts could have been analyzed by capillary GC without any additional sample preparation.

Conventional analysis of an agricultural soil containing native 2,4-D and dicamba was performed by a laboratory specializing in pesticide determination. A rigorous extraction method used was based on two sequential extractions of a soil sample with 0.5 *M*

KOH in 10% KCl/water first on a boiling water and then with mechanical shaking (in the procedure esters were hydrolyzed to their free acids for extraction into the basic water). To remove interferences the supernatants were washed with chloroform, acidified to pH < 1.5 and extracted, again with chloroform, to recover the pesticides (acid form). Before GC analysis acidic herbicides were derivatized with diazomethane.

For tests, samples of a river sediment (organic carbon content — 4%) showing no detectable levels of 2,4-D were spiked with 2,4-D in methanol — the solvent was evaporated before extraction. The real samples of pesticide-contaminated agricultural soil (silty clay from Montana with ca. 7% water) were analyzed as received.

In an initial step of the derivatization-SFE procedure development, clean sand spiked at 20 ppm with 2,4-D was treated with 1.5% TMAH reagent. Interestingly, varying the derivatization time (5 to 30 min at 100°C) or temperature (60 to 140°C for time 5 min) had no significant effect on 2,4-D recovery, which was essentially quantitative under all conditions.

An advantage of chemical derivatization techniques is their potential to selectively derivatize target analytes. For example, BF_3/methanol has been reported to efficiently methylate 2,4-D but not dicamba, while under the same conditions (80°C, 40 MPa) TMAH derivatized both herbicides. Good recoveries of spiked 2,4-D (98 ± 6%) were obtained from the sand; in the case of river sediment the recoveries dropped to only 23%. With three sequential derivatization-SFE steps the total recovery was higher than 90%. Additional experiments performed indicate that the presence of reactive matrix components (e.g., humic material) must be considered in selecting reagent concentrations and derivatization times in the *in situ* derivatization-SFE method. The tests performed on agricultural soil polluted with 2,4-D and dicamba showed that multiple derivatization-SFE is necessary to obtain relatively high recovery.

Croft et al.[32] conducted studies on rapid and efficient methylation and extraction of 2,4-D and 2,4,5-T herbicides from aqueous solutions and soils using supercritical CO_2 containing methyl iodide and tetrahexylammonium hydrogen sulfate.

For test experiments soil samples were spiked with 2,4-D at 1.3 ppm and 2,4,5-T at 0.5 ppm. Spiking was performed with great care to obtain uniform distribution of the spike; the spiked material was aged for approximately 1 month. The static supercritical CO_2 extraction coupled with methylation (tetrahexylammonium hydrogen sulfate (THA) solution and methyl iodide) were typically performed at a constant pressure of 20 MPa and a temperature of 60 to 90°C. Extracts from spiked soil were sufficiently clean to quantitate the methyl esters by GC-ECD. Recoveries were in the range of 60 to 70%.

An alternative to SFE is extraction under increased pressure or pressurized fluid extraction (PFE), also termed accelerated solvent extraction (ASE). PFE has the same advantages as SFE, that is, reduced extraction time and small solvent consumption and, additionally, is characterized by higher flexibility in the extraction mixture composition. Analyte recoveries are generally higher; in the case of some analytes much higher. In PFE changes of pressure and temperature are less important for the process.

Based on the agreement of the results of Soxhlet and PTE extractions of organochlorinated and organophosphorus pesticides and herbicides from different soils the Environmental Protection Agency (EPA) has evaluated PTE as equally good in performance as the Soxhlet extraction.[33,34]

David et al.[35] worked on the application of *in situ* derivatization coupled with PFE for the determination of 2,4-D, 2,4,5-T, dicamba, silvex, trichlopyr, and bentazone herbicides in soil. The effectiveness of this

approach was comparable with traditional extraction-*ex situ* derivatization procedures.

They conducted studies with the use of two environmental solid matrices: soil with 4.4% organic carbon and 86% clay and sand spiked with herbicides at 0.5 and 50 mg/kg. In the three PFE based procedures acidic extractant, *in situ* derivatization-extraction and Na_4EDTA assisted extraction were used.

In acidic extractant method soil was first extracted with a mixture of methylene chloride and acetone (1:2) containing 4% aqueous solution of phosphoric acid (1:1) and then the extract *ex situ* derivatized. In this approach PFBB derivatizing reagent was not effective.

In the *in situ* derivatization method with PFBB solution as a derivatizing reagent, and acetone as solvent, recoveries of six selected herbicides were quantitative at high and low concentrations from sand and at higher concentrations from soils. The method is then applicable to these matrices.

In initial studies Na_4EDTA addition was found out to increase recovery of 2,4-D from 31 to 94%. Admittedly, the recovery is comparable with that of traditional methods, but the method is simpler and shorter, and several samples can be extracted simultaneously.

For samples of sand spiked at 0.5 and soil at 0.5 and 50 mg/kg, average recoveries for six analytes were 107 for sand and 93 and 68% for soil.

For determination of chlorophenols in soils Wennrich et al.[36] used ASE combined with solid-phase microextraction. The studied chlorophenols included PCP, which is used as a herbicide. For the investigation of pollution with chlorophenols the soils collected were air-dried at room temperature, sieved to a grain size of <2 mm, homogenized, and stored at 4°C. All the studies to optimize the extraction procedure were performed using only a slightly polluted wetland soil, a sandy loamy silt with a total carbon content of 2.41% (humus content 4.14%).

The studies were done on ASE extraction from the soil spiked at concentrations of 1000, 100, 10, and 1 μg/kg of each chlorophenol. Water and aqueous solutions containing 3 or 5% (v/v) of an organic modifier, such as methanol, acetone, or acetonitrile were used for extraction.

The addition of organic solvents, especially acetone and acetonitrile, was found out to improve the extraction efficiency significantly, with the highest yields being obtained by adding 5% acetonitrile. Using diluted phosphoric acid (3%, w/v) as an extraction solvent for ASE was not successful.

Wennrich et al.[36] analyzed real soil samples using ASE-SPME based procedures and the EPA 3545 method as a reference method. They obtained better results in ASE when water modified with 5% acetonitrile than when organic solvents were used.

Santos-Delgado et al.[37] proposed a method for extraction and determination of 2,4-D in free acid and ester forms. Chlorophenoxyalkanocarboxilic acids were exctracted from soil with a mixture of ethyl ether and water and converted to methyl esters by fast reaction with CH_3OH in presence of H_2SO_4 as a catalyst. They used simplex method for parameters optimization.

Two soils were analyzed: soil I — inorganic matter – sand 67.5%, silt 20.1%, clay 12.4%; organic matter 0.7%; soil II — sand 74.5%, silt 20.3%, clay 4.3%; organic matter 0.4%. Recoveries for soil II were in the range of 85 to 97% and were always higher than for soil I (76 to 90%). This clearly shows that soil composition (content of organic matter and composition of inorganic matter) has noticeable effect on recovery.

III. SAMPLE PREPARATION FOR LIQUID CHROMATOGRAPHY

Soil or any other solid samples cannot also be directly introduced into a LC col-

umn, and herbicide analytes must be first isolated and transferred into a suitable solvent. The difference in GC and LC results from the fact that acidic herbicides do not have to be derivatized for LC analysis. For improving sensitivity and selectivity, they are sometimes converted to appropriate derivatives but of quite different properties from those for GC analysis.

In the method developed by Pozo et al.[38] for the determination of MCPA and its main metabolite 4-chloro-2-methylphenol in soil, the first step in sample preparation for LC-MS analysis was extraction with basic solvent (KOH aqueous solution). The extracts obtained were cleaned up by means of SPE (extraction disks packed with C_{18}-modified silica gel).

In fact, LC is used more often than GC for MCPA determination due mainly to the fact that a step of derivatization can be eliminated from sample preparation. The main obstacle to the LC use was lack of sufficiently sensitive and selective detectors for acidic herbicides. However, this problem is less and less critical as LC- (API) MS becomes increasingly common.

Pozo et al.[38] conducted the studies for two soils — a soil from a citrus orchard (0.8% organic matter) and a soil from a greenhouse (2.4% organic matter). Soil samples were air-dried, homogenized, and then spiked with analytes of interest and aged overnight. Acidic herbicides were extracted with aqueous solution of KOH. The clear liquid phase was acidified to pH = 2 to 3 and humic matter precipitate centrifuged. After SPE clean-up the final concentrate was analyzed by LC-MS-MS.

Meier et al.[39] worked on determination of phenoxy acid herbicides (2,4-D, MCPA, 2,4,5-T) by means of HPLC combined with sample preparation in off-line and on-line mode. They conducted studies for three different soils: weak humous soil (1); strong humous, loamy sand (2); weak humous, sandy loam (3). Soils whose water content was kept at 40% were spiked with phenoxy acids at two concentration levels: 0.5 to 5.0 ppm or 2.5 to 10.0 ppb. Herbicides were extracted with aqueous NaOH solution. HPLC with UV detection was used for final analysis. In the case of lower concentrations (to 2.5 ppb) the extract was analyzed by a coupled SPE-HPLC system (*on-line* enrichment).

Independently of soil kind and herbicides concentration, the highest recoveries were obtained for MCPA (for 2,4-D 68 to 92%, 2,4,5-T 60 to 83%, MCPA 72 to 89%). With an increase in the content of organic matter and clay fraction in soil, recoveries decreased. Precision was quite good for a wide range of concentrations.

Alonso et al.[40] compared two methods of extraction (Soxhlet extraction and MAE) of phenolic herbicides (pentachlorophenol and other chlorophenols and nitrophenols) from soil. Soil samples to be studied were lyophilized, sieved (120 μm screen), and homogenized before analysis.

Soxhlet extraction was rather long process (12 h) and consumed large quantities of solvent (100 ml). Some of the solvents studied (methanol, acetone-methanol, acetone-methyl chloride, methanol- methyl chloride, methanol-water, methanol-water with 2% triethylamine (TEA) and methanol-water with 1% acetic acid) are unfriendly to the environment.

Extracts were filtered through GF/F glass microfibres, cleaned up on styrene-divinylbenzene copolymer sorbent, and eluted with acetonitrile.

The eluates were sufficiently clean and concentrated to be analyzed by LC-UV and LC-MS (with atmospheric pressure chemical ionization).

The highest recoveries of phenolic compounds from soil were obtained when methanol-water extraction mixture containing 2% TEA was used. The presence of water in an extract was not a problem since RPLC was applied. MAE proved a good alternative to

conventional Soxhlet extraction. An extractant volume was lower by half, and extraction time only 30 to 40 min (for recoveries above 70%) compared with 12 h in Soxhlet extraction. Because the same extractant can often be used in MAE as in Soxhlet extraction and procedures developed for Soxhlet extraction can easily be adopted to MAE. The procedures based on MAE can be implemented to routine analysis.

Hogendoorn et al.[41] developed a screening method for the determination of acidic pesticides in various types of soil. In the method microwave-assisted solvent extraction was used for the fast and efficient isolation of the analytes from soils and coupled-column reversed-phase liquid chromatography (LC-LC) with UV detection at 228 nm for the instrumental analysis of uncleaned extracts.

The method was tested for 10 acidic pesticides of different chemical families (bentazone, bromoxynil, metsulfuron-methyl, 2,4-D, MCPA, MCPP, 2,4-DP, 2,4,5-T, 2,4-DB, and MCPB). The four soils: sand of Hulshorst (water –1.2%, organic matter –0.3%), clay of Houten (water –16.1%, organic matter –3.9%), peat-1 with 28.1% water and 10.4% organic matter, and peat-2 with 39.8% water and 12.9% organic matter. The freshly spiked samples were allowed to stand in the open bottles for 24 h at ambient temperature before extraction. Samples with aged residues were stored, after air-drying, in the dark for 120 days at about 4°C. The four types of soils were spiked with the 10 acidic pesticides at levels between 20 and 200 μg/kg. The method was validated by the analysis of freshly spiked samples and samples with aged residues (120 days). MAE was carried out in PTFE-lined extraction vessels. The concentrate was analyzed by LC.

IV. FINAL ANALYSIS

For the final determination of herbicides in extracts nearly exclusively chromatographic techniques are used, mainly gas chromatography (GC) and liquid chromatography (LC).

A. Gas Chromatography

Due to high resolution power, the availability of a wide spectrum of sensitive and selective detectors and reliability of the results produced, gas chromatography plays an important role in herbicide analysis. Moreover, when compared with LC, GC is relatively cheap, simple, and uses environmentally friendly mobile phase (inert gas). It is highly recommended for the determination of herbicides in inherently complex environmental samples, including soils and sediments.

Due to low volatility and a tendency to adsorb on different elements of measuring systems acidic herbicides must be converted to less polar and more volatile derivatives. In this case derivatization is based on replacing acidic hydrogens for non-polar groups. In the process less polar, more volatile and thermally stable derivatives are produced, which can give symmetrical GC peaks.

Detection limits are affected by analyte properties, separation power of a GC system, sensitivity and selectivity of the detector applied, interfering substances present in sample matrix and reagents, extraction yield, etc.

Lou et al.[24] studied detection limits (LOD) for a method of determination of acidic herbicides in agricultural soil. In the case of 1.5-g soil samples from which analytes were isolated and enriched by means of SbWE/SAX the detection limits were of the order of 0.05 to 0.5 ppm (μg/g soil) for GC-ECD, and 0.01 to 0.5 ppm for GC-MS (for 2,4-DP decrease in LOD is not observed because its MS spectrum does not contain intensive mass peaks).

Also, Chiang et al.[25] used GC-ECD (^{63}Ni) and GC-MS with an HP-5 capillary column

(25 m × 0.2 mm, d_f 0.33 μm) coated with cross-linked phenylmethylsiloxane (5% phenyl groups).

Tsukioka and Murakami[26] applied GC-MS in ion monitoring mode for final analysis of acidic herbicides. For separation they selected a wide-bore column (15 m × 0.53 mm) with chemically bonded OV-17, which was able to separate eight studied herbicides in 6 min. Attempts made with Ultrabond 20 *M* stationary phase were unsuccessful due to high background. Selected ions were the following: (*m*/*z* 267 for MCPB, 369 for TBA, 380 for MCP, 394 for MCPP, 400 for 2,4-D and dicamba, 434 for 2,4,5-T, 435 for triclopyr). Detection limits for 20-g samples of rice field soil were 0.5 μg/kg for MCPP, MCP, TBA and MCPB; 1.0 μg/kg for 2,4-D and triclopyr; 1.5 μg/kg for dicamba and 2,4,5-T.

In real samples of rice field soil and sediments, contents of MCP and 2,4-D were on the level of 0.25 to 3.0 μg/kg, in the soil collected close to a chemical laboratory — TBA, triclopyr, MCP and dicamba on the level of 9 to 100 μg/kg. The authors[26] made attempts to use GC-ECD, but the number of interfering peaks was generally much larger.

Ngan and Ikesaki[11] applied GC-ECD with two parallel columns: one (30 m × 0.53 mm, d_f 0.5 μm) coated with (35%-diphenyl)-dimethylopolysiloxane (Rt_x-35) and the second (30 m × 0.53 mm, d_f 1.5 μm) coated with (5%-diphenyl)-dimethylpolysiloxane (Rt_x-5). This approach has two important advantages: confirmation can be done without column change and analysis costs are lower than in the case of GC-MS. Reproducibility for the system with two columns is high. For higher concentrations rather GC-MS is recommended. For the nine herbicides studied detection limits are comparable to or better than in the case of the EPA-recommended methods. The detection limits with separation on a Rt_x-35 column ranged from 0.04 μg/kg for dinoseb to 5.00 μg/kg for mecoprop (exception is MCPA — 15.0 μg/kg) and correspondingly from 0.02 μg/kg to 3.00 μg/kg (exception is MCPA — 12.0 μg/kg), with separation on the Rt_x-5 column.

Rochette et al.[27] analyzed final concentrates obtained with the use of SFE and different ways of derivatization of 2,4-D (sililation, methylation, ion pairing, and ion exchange) by means of GC-ECD, with a DB-5 (5%-phenyl)-methylpolysiloxane)-coated capillary column.

Lopez-Avila et al.[30] used GC-MS for analysis of extracts. The injections were performed in the splitless mode into a PTE-5 fused silica capillary column (30 m × 0.25 mm i.d., d_f 0.25-μm). The quantification of the analytes was performed by internal standard calibration using acenaphthene-d_{10} and phenanthrene-d_{10}. They also made attempts to analyze the extracts from the TBA/MI experiments by GC-ECD; however, the presence of excess derivatizing reagent (MI) in the extracts interfered with GC-ECD determination.

Hawthorne et al.[31] performed all analyzes using a gas chromatograph equipped with an elector capture detector and an HP-5 capillary column (25 m × 0.25 mm, d_f 0.17 μm) coated with cross-linked phenylmethylsiloxane (5% phenyl groups). All SFE extracts could have been analyzed by capillary GC without any additional sample preparation. For quantification, an internal standard (1-chloronaphthalene) was added to extracts after the SFE step.

For the determination of yields of methyl ester formation, Croft et al.[32] used GC with ECD. Separation was carried out in an HP Ultra 1 column (12 m × 0.2 mm i.d., d_f 0.33 mm). To improve the detection limits of the procedure, the authors proposed reducing the final extract volume and cleaning up the sample before methylation via preextraction with supercritical CO_2 in the absence of methyl iodide.

David et al.[35] applied GC coupled with MS; separation was carried out with the use of a DB-5 ms column (30 m × 0.25 mm,

d_f 0.25 μm) coated with (5% phenyl) –95% methyl polysiloxane and a DB-17 ms column (30 m × 0.25 mm, d_f 0.25 μm) coated with 50% phenyl –50% methyl polysiloxane.

Wennrich et al.[36] used GC-MS (SIM) with a PTE-5 capillary column (30 m × 0.25 mm, d_f 0.25 μm) coated with poly(5% diphenyl/95% dimethylsil)oxane. Sample enrichment was based on ASE-SPME (solid phase microextraction). The SPME experiments were performed using a manual SPME device from Supelco with a 85 μm polyacrylate fiber. For SPME extraction the aqueous soil extracts were sodium chloride saturated and pH adjusted to 2.

Two ASE-SPME-GC-MS procedures without and with an organic modifier (5% acetonitrile) were evaluated with respect to precision and detection limits. For the former, the relative standard deviations (RSD) were in the range from 6.7% (2-CP) to 16.9% (2,3,4-TCP). Slightly higher RSD values ranging from 9.3% (2-CP) to 20.3% (PCP) were obtained for the latter. Detection limits in the low-ppb range were achieved using both procedures, with the LOD values being lower for the latter procedure (1.1 to 6.7 μg/kg). The reproducibility of replicate water extractions-SPME-GC-MS analysis (n=6) was in the range 7 to 20% RSD for the nine chlorophenols investigated. The tests were also made on real samples of five soils; the comparison with "classical" ASE procedures using organic solvents shows that subcritical water under subcritical conditions extracts chlorophenols more effectively from the soil matrix than the organic solvents used by the authors.[36]

For analysis of soil extracts for esters of phenoxy acids, including 2,4-D Santos-Delgado et al.[37] used GC-FID; for conformation of identification, GC-MS was used. The soils contained phenoxy acids at 0.5 to 5 μg/ml and a mixture of 2,4-D esters at 0.5 to 10 μg/ml. The detection limits for the complete procedure were 27 μg/l for phenoxy acids and 75 μg/l for 2,4-D esters. The precisions of measurements expressed as RSD were 4 to 7% for phenoxy acids at 5 μg/ml and 5 to 7% for 2,4-D esters at 10 μg/ml.

B. Liquid Chromatography

For analysis of nonvolatile and thermally labile compounds liquid chromatography can also be used; with this method acidic herbicides can be separated without derivatization necessary when GC is applied. A very attractive approach is the application of a LC-MS coupled system. With MS detection, high sensitivity and selectivity can be achieved, which diminishes qualitative and quantitative errors. Fragment ions, generally present, increase the reliability of identification.

For the determination of MCPA Pozo et al.[38] applied LC-MS-MS coupled systems equipped with different interfaces, including particle beam interface and thermospray. Recently, atmospheric pressure ionization (API), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI) have become increasingly popular. Pozo et al.[38] used a mixture of acetonitrile and 0.01% formic acid as a mobile phase.

They analyzed two soils containing MCPA at 0.005 and 0.05 mg/kg. Better accuracy (recovery in the range of 87 to 91%) was obtained for soil from a citrus orchard with a lower content of organic matter; for greenhouse soil, recoveries were in the range of 75 to 76%. The precissions expressed as RSDs were 7% and 10% for soil from the citrus orchard and greenhouse, respectively.

Meier et al.[39] determined phenoxy acid herbicides by means of HPLC with UV detection at 230 nm. In the case of a solution of 0.25 ppm herbicide concentration, 10 to 20 μl samples were directly injected into LC. For solutions of 2.5 ppb herbicide concentration 1-ml samples were injected via an on-line SPE enrichment system. A LC column was packed with a RP-18 stationary phase, the mobile phase was a mixture of methanol and

diluted phosphoric acid (pH = 2.5) in the case of direct injection. The detection limits for herbicides in soils were 0.2 ppm and 2 ppb for direct extract injection and via the on-line SPE enrichment system, respectively.

For final detection of phenolic herbicides in soil, Alonso et al.[40] applied LC coupled with APCI-MS and LC with UV detection. For both techniques extracts were prepared in the same way. With LC-UV detection limits were on the level of 100 ng/g for PCP. With LC-APCI-MS (SIM mode) detection limits were much lower; depending on a phenolic compound they ranged from 0.007 ng/g for PCP to 0.4 ng/g for 2-chlorophenol. The whole procedure was tested on soil reference materials and then applied to soils collected in Brazil where PCP was determined at 1660 ng/g.

The method developed by Hogendoorn et al.[41] included the selection of suitable MAE and LC-LC conditions. The LC-LC system consisted of a 5 μm GFF-II internal surface reversed-phase (ISRP) analytical column (50 × 4.6 mm i.d.) as the first column in the restricted access material (RAM)-C_{18} configuration in combination with an optimized linear gradient elution, including on-line cleanup of sample extracts and reconditioning of the columns.

The combination of MAE and a coupled column system RPLC-UV (228 nm) employing an analytical RAM column is a viable approach for the multiresidue screening of acidic pesticides of different chemical classes in various types of soils with a wide range of organic matter content.

In the case of freshly spiked samples, overall recoveries of the 10 different acidic pesticides were between 60 and 90% for most soil-pesticide combinations with reproducibility expressed as a relative standard deviation below 25%. LODs were between 5 and 50 μg/kg. Similar results were obtained for samples with aged residues, except for the peat soil with the highest organic matter content (12.9%). The recoveries from peat soil samples were quite low (11 to 33%) for bentazone, bromoxynil, 2,4-DB, and MCPB, and medium for the remaining compounds (average values between 45 and 75%).

Evaluation of the data set with principal component analysis revealed that the increase in organic matter content of soil and residues aging negatively affect the recovery of analytes.

V. RESUME

The necessity of determining low concentrations of acidic herbicides as well as the complexity of soil and sediment matrices mean that analytical methods of high sensitivity, selectivity, and separation power must be applied. Generally, elaborate sample preparation involving analytes isolation and enrichment is necessary before the final analysis, usually performed by gas or liquid chromatography. When using GC carboxylic acid herbicides must and phenolic herbicides should be converted to sufficiently volatile derivatives. Depending on the extraction and derivatizing reagent, derivatization is carried out directly on soil (*in situ*) or in a concentrate (*ex situ*). The former can be especially effective and convenient in combination with SFE and ASE, which are used most often in soil and sediment sample preparation for chromatographic analysis. For liquid chromatographic analysis soil samples are prepared in a manner similar to the case of GC analysis. However, derivatization is not necessary in the case of LC, although sometimes it is used to increase sensitivity and selectivity and, in this way, to improve detection limits. However, functional groups introduced to herbicide analytes are quite different from those used for GC separation. The results obtained and the extraction conditions used are strongly dependent on soil matrix — large content of organic matter

and clay fraction make extraction more difficult and decrease recoveries.

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