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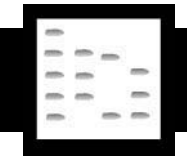
HERBICIDES



Gas Chromatography

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Herbicide Formulations

Weeds have been controlled by humans since the beginning of agriculture by means of mechanical tools or by hand. It was early in the 20th century that some inorganic compounds were first used with this aim. The discovery of the herbicidal properties of 2,4-D (2,4-dichlorophenoxyacetic acid) in 1945 can be considered the initiation of use of organic herbicides in agriculture. Since then, more than 130 different active compounds have been synthesized for their application as herbicides. These compounds can be grouped, according to their chemical structures, into different herbicide classes (**Table 1**).

Compounds belonging to the principal herbicide groups will be considered in this study. These compounds control weeds in a variety of ways, showing different modes of action, selectivity and application characteristics. Soil-applied herbicides are absorbed by roots or emerging shoots and foliage-applied herbicides are absorbed into the leaves, where they may be translocated to other parts of the plant.

The active ingredient of a herbicide is a compound, usually obtained by synthesis, which is formulated by a manufacturer in soil particles or liquid concentrates. These commercial formulations of herbicides are diluted with water before application in agriculture at the recommended doses. Herbicide formulations generally contain other materials to improve the efficiency of application.

Analysis of herbicide formulations was initially carried out by wet chemical procedures, such as determination of total chlorine, nitrogen or phosphorus, or by spectrometric procedures like ultraviolet absorption. The development of gas chromatography (GC) allowed the analysis of these compounds in commercial formulations with high selectivity and sensitivity. The analytical procedure is commonly based on the dissolution of a known amount of the formulation in an organic solvent, which often contains an internal standard to improve the precision and accuracy of the determination. An aliquot of this solution is analysed by GC. Packed columns were used initially, but have now been replaced by capillary columns of low or medium polarity and flame ionization is the detection technique more widely used. When herbicides are not volatile or thermally stable, high performance liquid chromatography (HPLC) is the preferred technique for their determination in commercial formulations. **Figure 1** shows the gas chromatographic separation of a mixture of phenoxy esters.

Herbicide Residue Analysis

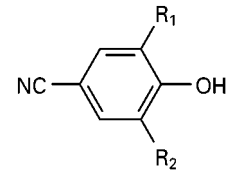
Residues of herbicides will persist in the plant or in the soil for a variable time, depending on their physicochemical properties and on the environmental conditions. Analysis of herbicide residues in these matrices is important, not only from the point of view of the efficacy of application, but also to know the distribution and persistence of these compounds in food and in the environment. Therefore, herbicides of a wide range of polarities have to be determined in complex environmental matrices at very low levels.

Initially, herbicide residues were analysed by colorimetric methods. These procedures were generally based on acidic or basic hydrolysis followed by formation of derivatives. These methods are time-consuming and do not usually distinguish between the parent herbicide and metabolites.

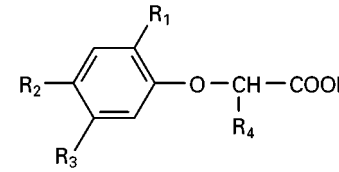
Since the development of GC, this technique has been widely used in the analysis of these compounds. **Table 2** summarizes the preparation of different types of samples for residue determination. These samples are generally analysed by a procedure with the following main steps: sample extraction, clean-up of extracts, then GC determination and identification. Some compounds are not volatile or thermally stable

Table 1 Chemical structures of herbicides

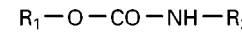
Benzonitriles
Bromoxynil; ioxynil



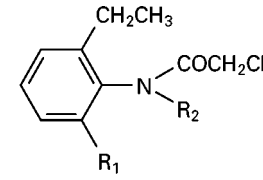
Phenoxyacids
2,4-D; MCPA; MCPP; dichlorprop; diclofop; fenoxaprop



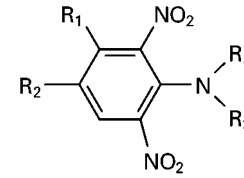
Carbamates
EPTC; triallate



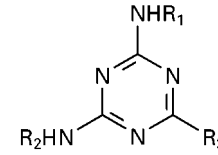
Chloroacetamides
Alachlor; metolachlor



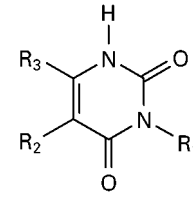
Dinitroanilines
Butralin; ethalfluralin; pendimethalin; trifluralin



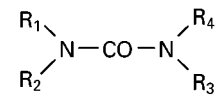
Triazines
Ametryn; atrazine; cyanazine; simazine; terbutryn



Uracils
Bromacil; lenacil; terbacil



Ureas
Chlorotoluron; isoproturon; linuron; chlorsulfuron; metsulfuron; triasulfuron



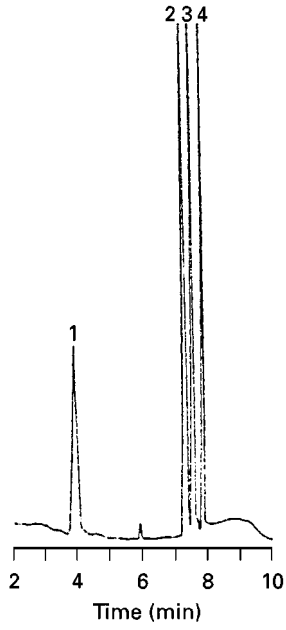


Figure 1 Analysis of a mixture of phenoxy esters by gas chromatography on a BP-5 fused silica column, 12 m × 0.53 mm i.d., with helium as carrier gas at 10 mL min^{-1} and flame ionization detection. Oven temperature was held at 180°C for 5 min, increased at 25°C min^{-1} to 250°C and held for 10 min. 1, 2,4-D isobutyl ester; 2, MCPA 2-butoxyethyl ester, 3, 2,4-DP 2-butoxyethyl ester; 4, 2,4-D 2-butoxyethyl ester. Adapted from Sánchez-Brunete C, Pérez S and Tadeo JL (1991) Determination of phenoxy ester herbicides by gas and high-performance liquid chromatography. *Journal of Chromatography* 552: 235 with permission from Elsevier Science.

and need to be derivatized before being analysed by GC.

Currently capillary columns are most commonly used for residue analysis. **Table 3** shows some representative examples of different packed and capillary columns used in the determination of herbicides.

In trace analysis, blank samples are commonly processed through the analytical method to identify the possibility of interferences in the herbicide determination from other sample or reagent components. In addition, recoveries of the analysed compound are also carried out through the extraction and clean-up procedures. The accepted normal range of these recoveries is 70–120%, with a standard deviation of 20%.

Several injection techniques are used in herbicide residue analysis. The techniques most often employed are splitless, on-column and programmed-temperature vaporizer injection and the volume normally injected is 1–2 μL.

Various selective detectors are used in trace analysis of herbicides. The electron-capture detector (ECD) was first used for the determination of halogen-containing compounds or halogenated derivatives, due to its high sensitivity for these compounds. The nitrogen–phosphorus detector (NPD), also known as thermionic or alkali flame detector, is commonly used in the analysis of nitrogen-containing herbicides. Both detectors are highly sensitive and can detect herbicide concentrations lower than 1 pg. The flame photometric detector (FPD) has sometimes been used in the determination of sulfur-containing herbicides. The limit of detection (LOD) reflects the sensitivity of a detector for a given compound and it is defined as the amount producing a signal-to-noise ratio equal to 3. When this ratio is determined with extracts of real samples, processed through the whole analytical procedure, this parameter is known as the limit of quantification (LOQ), which depends on the efficacy of the extraction and clean-up procedures and on the selectivity and sensitivity of the detector. The coupling of mass spectrometry (MS) with GC allows the determination of herbicide residues with high selectivity and sensitivity and, in addition, the identification of residues by means of their mass spectra obtained at trace levels. The atomic emission detector allows monitoring characteristic wavelengths for carbon and hydrogen atoms, as well as the more specific emission lines for phosphorus, nitrogen and sulfur. **Table 4** summarizes the detection techniques for herbicide residue determination by GC.

The analysis of herbicides, grouped into various chemical classes, is considered in more detail below.

Benzonitriles and Phenoxy Acids

Benzonitriles and phenoxy acids are widely applied as salts or esters, but they are hydrolysed to their

Table 2 Procedures used in sample preparation for herbicide residue determination

Matrix	*Amount sampled*	*Sample preparation*	*Amount extracted*	*Extraction procedure*
Soil	1 kg	Sieving (< 2 mm)	10–20 g	Shaking, Soxhlet, SFE
Water	1 L	Filtration	0.1–1 L	SPE, LLE
Plants	1 kg	Blending	20–50 g	Homogenization
Air	25–250 L	Adsorption or trapping		Thermal or solvent desorption

SFE, Supercritical fluid extraction; SPE, solid-phase extraction; LLE, liquid–liquid extraction.

Table 3 Chromatographic columns used in herbicide residue analysis

Column (length/diameter)	*Stationary phase*	*Applications*
Packed (1–3 m/2–4 mm)	Dimethylpolysiloxane (SE-30, DC-200)	Carbamates, ureas, dinitroanilines, triazines
	Phenylmethylpolysiloxane (OV-17, OV-25)	Benzonitriles, phenoxy acids
	Trifluoropropylpolysiloxane (QF-1, OV-210)	Carbamates, chloroacetamides, phenoxy acids
	Polyethyleneglycol (Carbowax)	Triazines
	Cyanoethylpolysiloxane (XE-60)	Triazines
	Cyanopropylpolysiloxane (OV-225)	Ureas
Capillary (10–30 m/0.2–0.5 mm)	Dimethylpolysiloxane	Nitrogen-containing herbicides
	Phenylmethylpolysiloxane	Triazines
	Polyethylene glycol	Triazines, phenoxy acids, benzonitriles
	Cyanopropylphenylmethylpolysiloxane	Multiresidue

respective phenols or acids in the matrix. Extraction of residues from soil and water is commonly performed at acidic pH with organic solvents of medium polarity. The extraction of these herbicides from vegetable matter is often done with aqueous solutions at basic pH, followed by extraction with organic solvents.

Purification of extracts is required in most cases and this step is accomplished by liquid–liquid partition at basic pH or by chromatography on silica columns.

Analysis of these compounds in air is carried out by trapping herbicides in ethylene glycol or in various adsorbents, like polyurethane or amberlite resins.

Derivatization of phenoxy acids, before GC determination, is necessary to make them volatile. Various alkyl, silyl or pentafluorobenzyl derivatives are obtained with this aim. Methyl esters have been commonly prepared for the determination of phenoxy acids and the reagents most often used are diazomethane and boron trifluoride–methanol. Benzonitriles can be determined directly by GC, but the sensitivity and reproducibility achieved are poor. Various derivatives overcome these problems and diazomethane and heptafluorobutyric anhydride are the reagents most often used.

The determination of herbicides is widely carried out by GC with ECD, if the compound has halogen substituents or halogenated derivatives are obtained. MS detection has the advantage of being more selective and requiring less clean-up of extracts.

Carbamates

Carbamates are a wide group of pesticides and some of them have herbicide properties, like the thiocarbamates S-ethyl dipropylthiocarbamate (EPTC) and triallate. These compounds are extracted from soil with methanol or acetone and from water by means of hexane or dichloromethane. The extraction from plants is commonly accomplished with acetonitrile or by steam distillation. Clean-up of extracts is often

Table 4 Detection of herbicides in environmental samples

Herbicides	*Detectors*	*LOD* ($\mu g\, g^{-1}$)	*Derivatives*
Benzonitriles	ECD, MS	0.05–0.0003	Methyl ethers
	MS	0.001	Heptafluorobutyryl
Phenoxyacids	ECD, MS	0.05–0.001	Methyl esters
Carbamates	ECD, NPD, FPD, MS	0.1–0.001	
Chloroacetamides	NPD, MS	0.05–0.001	
Dinitroanilines	ECD, NPD, MS	0.05–0.0001	
Triazines	NPD, ECD, MS	0.01–0.0001	
Uracils	NPD, ECD, MS	0.04–0.001	
Ureas			
Phenylureas	NPD, MS	0.1–0.01	Methyl or ethyl
	ECD	0.01–0.001	Heptafluorobutyryl
Sulfonylureas	ECD	0.1–0.002	Methyl or PFB
Multiresidue	NPD, MS	0.02–0.001	

ECD, Electron capture detector; MS, mass spectrometry; NPD, nitrogen–phosphorus detector; FPD, flame photometric detector; PFB, pentafluorobenzyl.

done by chromatography on silica columns. Most carbamates are thermally unstable and are usually analysed by HPLC. Some carbamates, such as the thiocarbamates considered above, can be determined by GC and their residues are determined by this technique using different detectors, like ECD, NPD, FPD and MS.

Chloroacetamides

These compounds, also known as anilides, are very often used for weed control in maize, in combination with triazines. Chloroacetamides are extracted from soil by polar and medium polarity solvents and their determination is generally carried out without further purification. Extraction from water is performed by reversed-phase solid-phase extraction (SPE) or by liquid–liquid extraction with low polarity solvents and clean-up of extracts on silica columns is necessary in some cases.

Analysis of these herbicides in plants is done by extraction with polar solvents, followed by purification by liquid–liquid partition or column chromatography on silica or alumina adsorbents.

Analytical methods for the determination of these herbicides in air have been reported, using several adsorbents or an ethylene glycol phase for their extraction from air. NPD and MS are the detectors most widely used in the determination of chloroacetamides; the ECD is also sometimes used.

Dinitroanilines

Dinitroaniline herbicides are highly lipophilic with a low solubility in water and some compounds have a remarkable volatility. Extraction of these compounds from soil is carried out by polar as well as by low polarity solvents. Dinitroanilines are extracted from water by SPE or by liquid–liquid extraction with low polarity solvents, such as dichloromethane. Clean-up of water and soil extracts on silica columns is sometimes needed.

Extraction with polar solvents, like methanol, is the method often used for the determination of dinitroanilines in plants, followed by liquid–liquid partition or Florisil column clean-up of extracts.

Analysis of these herbicides in air is performed by means of several adsorbents or organic solvents as trapping phases to remove these compounds from air.

ECD is often used in the determination of these compounds due to the large response obtained, particularly with some halogen-containing dinitroanilines. NPD and MS are also used in the gas chromatographic analysis of these herbicides.

Triazines

These compounds form a wide group of herbicides often employed in fruit trees and cereals; in particular, simazine and atrazine are the most widely used triazines.

Triazine herbicides are extracted from soil by polar or medium polarity organic solvents, followed by liquid–liquid partition or column chromatography clean-up if necessary. Supercritical fluid extraction is also used in the analysis of triazines in soil.

These herbicides are extracted from water by reversed-phase SPE, by anion exchange columns or by liquid–liquid extraction with low polarity solvents, followed in some cases by Florisil column clean-up.

Analysis of these compounds in plants is performed by sample homogenization with polar organic solvents and column chromatography or liquid–liquid partition clean-up. Triazines are sometimes analysed in air and plugs of polyurethane foam have been used to extract these compounds from air.

The gas chromatographic determination of triazines is commonly performed with NPD, due to the high response obtained with this detector because of the number of nitrogen atoms in their molecules. ECD is also employed but its sensitivity and selectivity for these compounds are lower. GC-MS is used for confirmation of residues as well as for routine determination, due to the good sensitivity obtained with selected ion monitoring. **Figure 2** shows some chromatograms of the determination of various triazines, together with other herbicides, by GC with NPD and MS detection.

Uracils

These compounds, also named pyrimidines, are used to control weeds in some fruit trees, vegetables and sugar beet. Extraction of these herbicides from soil is carried out with polar organic solvents or with basic aqueous solutions. Extraction from water is performed by SPE or by liquid–liquid extraction with low polarity solvents. Uracils are extracted from plants by homogenization with basic aqueous solutions or with mixtures of polar solvents with water. Clean-up of extracts is commonly carried out by liquid–liquid partition at different pHs. The gas chromatographic determination of these herbicides is performed using ECD and NPD detectors, and also by MS and atomic emission detection.

Ureas

Substituted ureas constitute one important group of herbicides formed by two different classes of compounds: phenylureas, one of the first used herbicides, and sulfonylureas, introduced later and applied

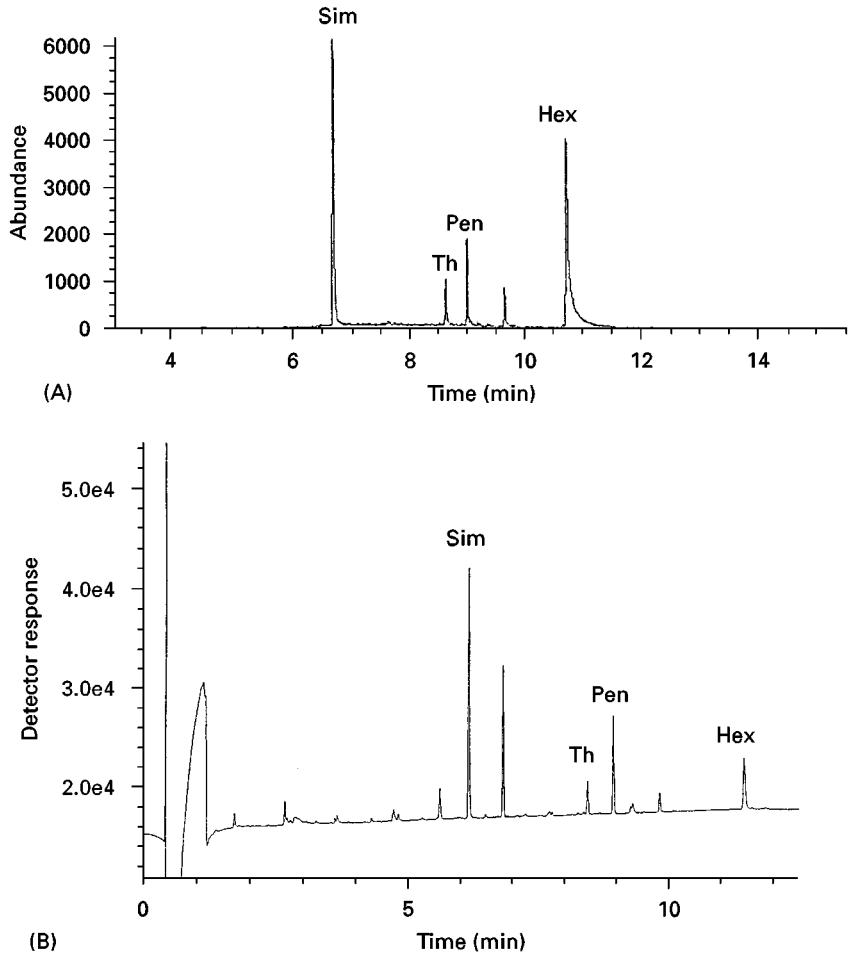


Figure 2 Gas chromatograms of herbicide residues in soil, fortified at 0.01 μg g^{-1}, separated on an HP-1 capillary column, 12.5 m × 0.20 mm i.d., with helium as carrier gas at 1 mL min^{-1} and detected (A) by GC-MS with selected ion monitoring or (B) by GC-NPD. Oven temperature was maintained at 100°C for 1 min, programmed at 15°C min^{-1} to 250°C and held for 1 min. Sim, simazine; Th, thiazopyr; Pen, pendimethalin; Hex, hexazinone. Adapted with permission from Pérez RA, Sanchez-Brunet C, Miguel E and Tadeo JL (1998) Analytical methods for the determination in soil of herbicides used in forestry by GC-NPD and GC-MS. *Journal of Agriculture and Food Chemistry* 46: 1864.

at lower doses due to their high herbicidal activity. Both types of compounds suffer from thermal instability and their decomposition products, rather than the parent compounds, have been determined in some cases. To overcome this problem, various derivatives amenable to GC have been obtained, mainly their methyl, heptafluorobutyryl or perfluorobenzyl derivatives.

Substituted ureas are extracted from soil by shaking with organic solvents of medium or high polarity, like methanol or acetone, sometimes followed by silica column or liquid–liquid partitioning clean-up. These herbicides are extracted from water by reversed-phase SPE or by liquid–liquid extraction with dichloromethane.

Urea herbicides are generally extracted from plants by homogenization with polar organic solvents. Supercritical fluid extraction of these compounds from plants has also been reported. Clean-up of extracts through column chromatography or liquid–liquid partition is necessary before GC determination.

Detection of these herbicides is performed by NPD or by ECD, mainly when halogenated derivatives are obtained. MS is also used in the determination of substituted ureas in environmental matrices.

Multiresidue Analysis

The wide range of polarities and physico-chemical properties of herbicides does not allow the determination of the more than 130 available herbicides in one analytical method. Nevertheless, it is advisable to use analytical procedures that allow the determination of as many herbicides as possible in one method. These multiresidue methods permit reduction in time and cost of analysis as well as detection of the possible

presence of herbicide residues in samples with unknown origin or contamination. A multiresidue method is able to determine all the herbicides that can be extracted, cleaned up, separated and detected in the conditions used in the analytical procedure. In some cases, two detectors – generally ECD and NPD – are connected at the end of the same chromatographic column to allow the detection of a wider type of compounds. MS is used as a universal detector and also for residue identification purposes. Atomic emission detection is a more recent detection technique which is increasingly used in trace analysis.

Herbicide residues are extracted from soil and plants with medium or high polarity solvents, like methanol, ethyl acetate and acetone. Vegetable samples are generally homogenized with organic solvent and soil samples are normally shaken and filtered. Matrix solid-phase dispersion (MSPD) is a technique which has recently been employed for pesticide residue determination in food samples: it performs sample extraction and purification at the same time. A simple multiresidue method, based on soil extraction in small columns, has been reported. **Figure 3** shows representative chromatograms of the analysis of various nitrogen-containing herbicides by this method.

Analysis of herbicides in water is generally based on liquid–liquid extraction with dichloromethane or on SPE using reversed phase columns, mainly C_{18}. Determination of herbicide residues in air is

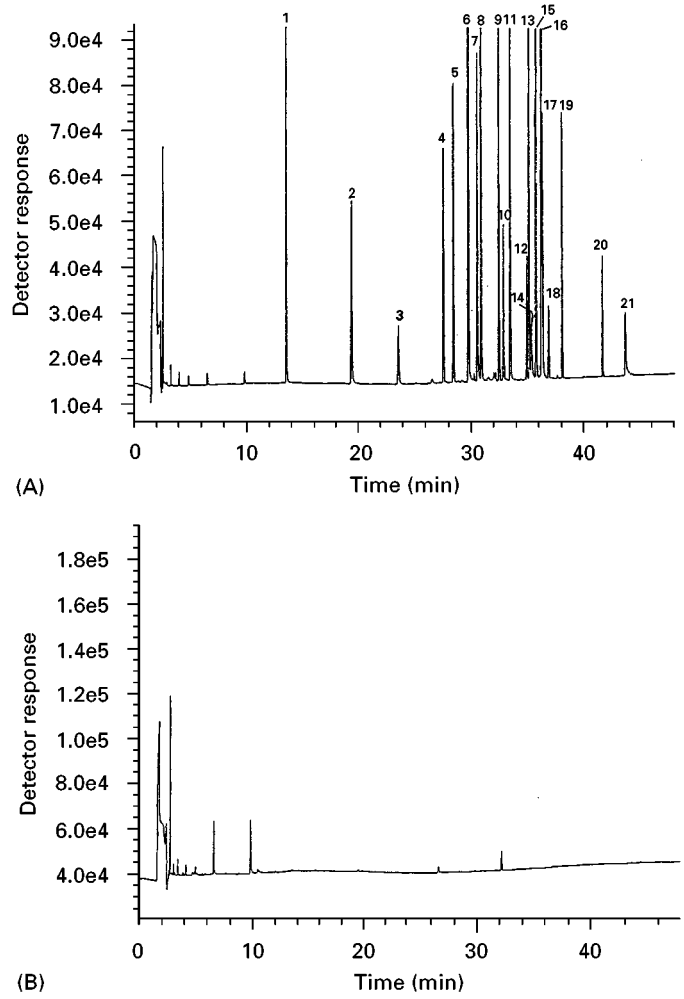


Figure 3 Multiresidue analysis of soil extracts separated on an HP-1 column, 30 m × 0.25 mm i.d., with helium as carrier gas at 1 mL min^{-1} and detected by GC-NPD. Oven temperature was kept at 80°C for 1 min, programmed at 5°C min^{-1} to 140°C, held for 10 min and programmed at 5°C min^{-1} to 250°C, held 15 min. (A) Soil fortified with nitrogen-containing herbicides at 0.5 μg g^{-1}. (B) Blank soil. 1, EPTC; 2, molinate; 3, propachlor; 4, ethalfluralin; 5, trifluralin; 6, atrazine; 7, terbumeton; 8, terbuthylazin; 9, dinitramine, 10, triallate; 11, prometryn; 12, alachlor; 13, metribuzin; 14, bromacil; 15, terbutryn; 16, cyanazine; 17, thiobencarb; 18, metolachlor; 19, butralin; 20, oxadiazon; 21, lenacil. Reproduced from Sánchez-Brunete C, Pérez RA, Miguel E and Tadeo JL (1998) Multiresidue herbicide analysis in soil samples by means of extraction in small columns and GC with NPD and MS detection. *Journal of Chromatography A* 823: 17, with permission from Elsevier Science.

accomplished by trapping these compounds on adsorbents, followed by extraction with organic solvents.

Future Developments

GC will continue to be the main chromatographic technique used in herbicide residue analysis in the near future, due to the high sensitivity and selectivity given by the detectors that can be coupled with this technique. In particular, the use of less expensive and more robust and sensitive GC-MS equipment will keep growing in the routine determination and confirmation of herbicide residues.

The time needed for sample processing is expected to be reduced as a consequence of the continuation in the development of automatic processes for sample preparation, extraction and clean-up. These processes will use less sample and lower volumes of organic solvents in the analytical procedure.

New improvements in the gas chromatographic equipment to allow higher injection volumes of less purified extracts can also be expected.

See Colour Plate 85.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **Insecticides:** Gas Chromatography. **Pesticides:** Supercritical Fluid Chromatography; Gas Chromatography. **Solid-Phase Matrix Dispersion: Extraction. III/Sorbent Selection for Solid-Phase Extraction.**

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Solid-Phase Extraction

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Solid-phase extraction (SPE) methods, using bonded-silicas, were first introduced in 1971 as an alternative to liquid partitioning. The method combines extraction and preconcentration of organic compounds in water by adsorption on proper solid material followed by desorption with a small quantity of an organic solvent. In comparison with liquid–liquid extraction, the following advantages are offered: the amount of solvent required for the clean up is greatly reduced, thus saving time for the evaporative concentration step and minimizing exposure of the analyst to the toxic solvent; the final eluate has less interfering material, and it could be analysed using any of a variety of detection, separation and identification techniques, including high performance liquid chromatography (HPLC) or gas chromatography (GC); accuracy and precision are improved; and it is rapid and easily automated.

Another impressive feature of SPE is the commercial availability of sorbents in small and inexpensive cartridges. C_{18}-bonded silica cartridges, styrene-divinylbenzene Empore® extraction discs and Carbopack® cartridges have been extensively used for the extraction of organic molecules from water samples. Automated column switching systems and on-line SPE coupled to determination devices have also been often reported for determination of pollutants in drinking and surface water.

Because of the reasons given above, in recent years much analysis of herbicides in fruit, vegetable and water has been conducted using SPE. Phenoxy acids, phenylureas, aryloxyphenoxypropionic acids, triazines, sulfonylureas, imidazolinones, glyphosate, phenoxyacetic acids, bipyridynium compounds,