an extract (from production) a day, yielding C_{60} with high purity. The relatively short time of SEC runs allows the frequency of injections to be increased with respect to other LC techniques.

This kind of automated system has also been used to separate and isolate metallofullerenes from empty cage fullerenes in sufficient amounts, despite their low concentration in the production mixtures (e.g. 200 mg of metallofullerenes isolated in 16 h).

However, the fact the C_{60} is eluted before C_{70} and other higher fullerenes, regardless of the mobile phase used (e.g. toluene, $CHCl_3$), means that solutes are not separated according to their size. Non-size effects (due to adsorptions and other types of interactions) have been described in the case of other relatively small molecules (e.g. PACs) with relative molecular masses lower than 1000.

Further Trends

Research is now focused on finding more selective stationary phases (mainly based on charge-transfer chromatography) to improve fullerene separation. An efficient method for separating individual fullerenes on a large (preparative) scale is still required. Most of the separation methods reported here are limited to gram scale. This has hampered the study of higher molecular mass fullerenes.

Further Reading

Ajie H, Alvarez MM, Anz SJ *et al.* (1990) Characterization of the soluble all-carbon molecules C_{60} and C_{70}. *Journal of Physical Chemistry* 94: 8630–8633.

Coutant DE, Clarke SA, Francis AH and Meyerhoff ME (1998) Selective separation of fullerenes on hydroxyphenyl-triphenylporphyrin-silica stationary phases. *Journal of Chromatography A* 824: 147–157.

Gross B, Schurig V, Lamparth I and Hirsch A (1997) Enantiomer separation of [60]fullerene derivatives by micro-column high-performance liquid chromatography using (R)-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy) propionic acid as chiral stationary phase. *Journal of Chromatography A* 791: 65–69.

Hirsch A (1994) *The Chemistry of the Fullerenes*. Stuttgart: Georg Thieme-Verlag.

Kimata K, Hirose T, Moriuchi K *et al.* (1995) High-capacity stationary phases containing heavy atoms for HPLC separation of fullerenes. *Analytical Chemistry* 67: 2556–2561.

Pirkle WH and Welch CJ (1991) An unusual effect of temperature on the chromatographic behavior of buckminsterfullerene. *Journal of Organic Chemistry* 56: 6973–6974.

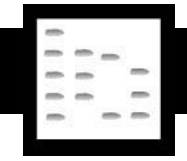
Scrivens WA, Cassell AM, North BL and Tour JM (1994) Single column purification of gram quantities of C_{70}. *Journal of the American Chemical Society* 116: 6939–6940.

Taylor R, Hare JP, Abdul-Sada AK and Kroto HW (1990) Isolation, separation and characterisation of the fullerenes C_{60} and C_{70}: the third form of carbon. *Journal of Chemical Society, Chemical Communications* 20: 1423–1425.

Theobald J, Perrut M, Weber JV, Millon E and Muller JF (1995) Extraction and purification of fullerenes: a comprehensive review. *Separation Science and Technology* 30: 2783–2819.

Welch CJ and Pirkle WH (1992) Progress in the design of selector for buckminsterfullerene. *Journal of Chromatography* 609: 89–101.

FUNGICIDES



Gas Chromatography

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Fungicides, a class of pesticides, are toxic substances that are used to prevent or kill the growth of fungi which are hazardous for plants, animals and human beings. Most fungicides for agricultural use are fumigated or sprayed over seeds, leaves or fruits to control and avoid a variety of economically important fungal diseases. The first fungicide of proven efficiency was the Bordeaux mixture, developed in 1882. This is a mixture of lime and copper(II) sulfate that has been used for a long time; nowadays a wide variety of compounds are used in a more selective way to fight specific fungi in specific plants. It is necessary to pay attention to their environmental impact (on water, the atmosphere, soil and food) and also to their presence in vegetables which are intended for direct human consumption. Among the important fungicides are those which are applied in greenhouses and in wine production.

There are two options for fungicide analysis: the determination of the composition of formulations, where the concentrations are relatively high, and the

evaluation of residues which appear after their use. Theoretically, it is assumed that the correct use of fungicides does not imply problems with residue because they should always be applied at nonhazardous levels. New pesticides are being developed with the aim of greater efficiency and lower environmental risk.

In formulation analysis there are not many analytical problems. In most cases high performance liquid chromatography (HPLC) is successfully applied. In some cases UV-Vis spectrophotometry or gas chromatography (GC) is used. In contrast, residue determination presents serious problems not only because it is focused on trace levels but also because the compound is usually found with other compounds or degradation products, and in a matrix that can present problems. This implies that the analyst must pay attention to all the steps in the analytical method from sampling to the interpretation of results. According to the problem the analyst will choose the best technique. This choice will affect other steps, especially which sample treatment is selected to achieve the lowest quantity of possible interference, good reproducibility and high recoveries. Sensitivity and selectivity always appear as opposed criteria, so it is necessary to balance the chance of a better signal-to-noise ratio against the risk of incompletely determining all the residues present.

There are three types of required analyses: the global determination of a group of compounds, e.g. ethylenbis(dithiocarbamate) (EBDCs); individual determinations; and, separation and quantification of chiral compounds. The method of analysis will vary according to the objective.

Table 1 summarizes common fungicides. The trend is to use several compounds of the same or a different chemical family, with the aim of achieving several modes of action over the development of the fungi. Because of this, a number of methods are devoted to the determination of several compounds in the same sample. Not all fungicides are shown in the table: inorganic materials such as sulfur, borax, mercury salts, arsenic and copper salts have been omitted. Recently developed compounds whose efficacy has not yet been proved or which are not registered in many countries, or whose mode of action is still unknown, are also omitted. As can be observed, there are a number of chemical families, the most important being phthalimides, benzimidazoles, dicarboximides, carbamates, sulfamides, anilinopyrimidines and phenylpyrrols. The nitrogen atom is common to all of them. In several cases there is a halogen atom and in fewer cases, sulfur or phosphorus atoms are included.

Technique Selection

Chromatographic techniques are the common choice for determining pesticide residues, and among them GC is useful for analysing organochlorine and organophosphorus residues because they have good thermal stability, they are volatile and they have a strong hydrophobic character. That this is valid for a great number of fungicides can be appreciated by the entries in the last column in **Table 1**. Nevertheless the thermal instability of some groups – EBDCs and benzimidazoles particularly – make the use of HPLC more appropriate, although it is also possible to produce derivatives that allow their determination by GC.

Column

Due to the large range of polarities of fungicides, it is difficult to select just one specific column, but many compounds can be separated using the mid-polarity columns. In several official methods for fungicide, glass columns (185 cm × 4 mm i.d.) packed with OV-101 or similar, on Chromosorb WHP (80–100 mesh) are still recommended, although nowadays fused silica open tubular capillary columns (FSOT) are preferred with lengths up to 60 m, inner diameter 0.2–0.7 mm and film thickness between 0.15 and 0.5 μm (DB-35, DB-1301, DB-1701 or similar). In commercial catalogues or application notes, many examples of fungicide separations are shown, together with the main chromatographic conditions and the equivalence between the different manufacturers.

Detectors

From the molecular formulae shown in Table 1, it is clear that the use of a nitrogen–phosphorus detector (NPD) is advantageous, although it is not as stable as the flame ionization detector (FID) and because of that it must be calibrated frequently. The electron-capture detector (ECD) is useful for many compounds, but in several cases, the co-extracted compounds can interfere with its response. As there are fungicides that have S and/or P atoms, the flame photometric detector (FPD) is also useful. Confirmation of identity can be obtained using two columns, one a 5% phenyl 95% methylsilicone bonded-phase column coupled to ECD and NPD and the other a 50% phenyl 50% methylsilicone column coupled to ECD and FPD. Such a system is very versatile and sensitive, allowing easy identification of the eluted compounds. Nevertheless, the best option to confirm compound identity is the use of coupled techniques such as gas chromatography–mass spectrometry (GC–MS); the specificity of GC–MS provides low

Table 1 Chemical group, molecular formula and recommended method of residue analysis of the most common fungicides

Fungicide	*Chemical group*	*Molecular formula*	*Method for residues*
Anilazine	Triazine	$C_9H_5Cl_3N_4$	GC, HPLC
Benalaxyl	Acylalanine	$C_{20}H_{23}NO_3$	HPLC, GC
Benomyl	Benzimidazole	$C_{14}H_{18}N_4O_3$	HPLC
Bitertanol	Azole	$C_{20}H_{23}N_3O_2$	GC
Bromuconazole	Azole	$C_{13}H_{12}BrCl_2N_3O$	GC
Bupyrimate	Pyrimidine	$C_{13}H_{24}N_4O_3S$	GC, HPLC
Captafol	Phthalimide	$C_{10}H_9Cl_4NO_2S$	GC
Captan	Phthalimide	$C_9H_8Cl_3NO_2S$	GC
Carbendazim	Benzimidazole	$C_9H_9N_3O_2$	HPLC
Carboxin	Phenylamide	$C_{12}H_{13}NO_2S$	GC
Chlorothalonil	Methoxybenzene	$C_8Cl_4N_2$	GC
Chlozolinate	Phthalimide	$C_{13}H_{11}Cl_2NO_5$	GC
Cymoxanyl	Acetamide	$C_7H_{10}N_4O_3$	GC
Cyproconazole	Azole	$C_{15}H_{18}ClN_3O$	GC
Cyprodinil	Pyrimidine	$C_{14}H_{15}N_3$	HPLC
Dichlofuanid	Sulfamide	$C_9H_{11}Cl_2FN_2O_2S_2$	GC
Diclomezine	Pyridazinone	$C_{11}H_8Cl_2N_2O$	GC
Dicloran	Nitrobenzamine	$C_6H_4Cl_2N_2O_2$	GC
Diethofencarb	Carbamate	$C_{14}H_{21}NO_4$	GC
Difenoconazole	Azole	$C_{19}H_{17}Cl_2N_3O_3$	GC
Dimetomorph	Morpholine	$C_{21}H_{22}ClNO_4$	GC, HPLC
Diniconazole	Azole	$C_{15}H_{17}Cl_2N_3O$	GC
Dinocap	Dinitrophenol	$C_{18}H_{24}N_2O$	GC
Diphenylamine	Amine	$C_{12}H_{11}N$	GC
Dodemorph	Morpholine	$C_{18}H_{35}NO$	GC
Edifenphos	Organophosphorus	$C_{14}H_{15}O_2PS_2$	GC
Ethirimol	Pyrimidine	$C_{11}H_{19}N_3O$	HPLC/GC
Etridiazole	Azole	$C_5H_5Cl_3N_2OS$	GC
Fenarimol	Pyrimidine	$C_{17}H_{12}Cl_2N_2O$	GC
Fenfuran	Carboxamide	$C_{12}H_{11}NO_2$	GC
Fenpliconil	Pyrrole	$C_{11}H_6Cl_2N_2$	HPLC
Fenpropidin	Morpholine	$C_{19}H_{31}N$	GC, HPLC
Fenpropimorph	Morpholine	$C_{20}H_{33}NO$	GC, HPLC
Ferbam	Dithiocarbamate	$C_9H_{18}FeN_3S_6$	HPLC
Fludioxonil	Phenylpyrrole	$C_{12}H_6F_2N_2O_2$	GC
Fluoroimide	Phenylpyrrole	$C_{10}H_4Cl_2FNO_2$	GC
Flusilazole	Azole	$C_{16}H_{15}F_2N_3Si$	GC, HPLC
Flutolanil	Phenylamide	$C_{17}H_{16}F_3NO_2$	GC
Flutriafol	Azole	$C_{16}H_{13}F_2N_3O$	GC
Folpet	Phthalimide	$C_9H_4Cl_3NO_2S$	GC
Fosetyl	Organophosphorus	$C_6H_{18}AlO_9P_3$	GC
Hexaconazole	Azole	$C_{14}H_{17}Cl_2N_3O$	GC
Hymexazol	Azole	$C_4H_5NO_2$	GC
Iprodione	Phthalimide	$C_{13}H_{13}Cl_2N_3O_3$	HPLC,GC
Imazalil	Azole	$C_{14}H_{14}Cl_2N_2O$	GC
Mancozeb	Dithiocarbamate	$(MnZnSCNH)_x$	HPLC
Maneb	Dithiocarbamate	$C_4H_6MnN_2S_4$	HPLC
Mepanypirim	Pyrimidine	$C_{14}H_{13}N_3$	GC
Mepronyl	Carboxamide	$C_{17}H_{19}NO_2$	GC
Methalaxyl	Phenylamide	$C_{15}H_{21}NO_4$	GC
Metiram	Dithiocarbamate	$(C_{16}H_{33}N_{11}S_{16}Zn_3)_x$	HPLC
Myclobutanyl	Azole	$C_{15}H_{17}ClN_4$	GC
Nabam	Dithiocarbamate	$C_4H_6N_2Na_2S_4$	HPLC
Nuarimol	Pyrimidine	$C_{17}H_{12}ClFN_2O$	GC
Ofurace	Phenylamide	$C_{14}H_{16}ClNO_3$	GC
Oxadixyl	Phenylamide	$C_{14}H_{18}N_2O_4$	GC
Oxycarboxin	Carboxamide	$C_{12}H_{13}NO_4S$	GC
Penconazole	Azole	$C_{13}H_{15}Cl_2N_3$	GC
Phthalide	Benzofuranone	$C_8H_2Cl_4O_2$	GC
Prochloraz	Azole	$C_{15}H_{16}Cl_3N_3O_2$	GC
Procymidone	Carboximide	$C_{13}H_{11}Cl_2NO_2$	GC
Propamocarb.HCl	Carbamate	$C_9H_{21}Cl\ N_2O_2$	GC

Table 1 *Continued*

Fungicide	*Chemical group*	*Molecular formula*	*Method for residues*
Propiconazole	Azole	$C_{15}H_{17}Cl_2N_3O_2$	GC
Propineb	Dithiocarbamate	$(C_5H_8N_2S_4Zn)_x$	HPLC
Pyrazophos	Organophosphorus	$C_{14}H_{20}N_3O_5PS$	GC
Pyrifenox	Oxime	$C_{14}H_{12}Cl_2N_2O$	GC, HPLC
Pyrimethanyl	Pyrimidine	$C_{12}H_{13}N_3$	HPLC
Tebuconazole	Azole	$C_{16}H_{22}ClN_3O$	GC
Tetraconazole	Azole	$C_{13}H_{11}Cl_2F_4N_3O$	GC, HPLC
Thiabendazole	Benzimidazole	$C_{10}H_7N_3S$	HPLC
Thiophanate methyl	Benzimidazole	$C_{12}H_{14}N_4O_4S_2$	GC, HPLC
Thiram	Thiocarbamate	$C_6H_{12}N_4S_4$	HPLC
Triadimefon	Azole	$C_{14}H_{16}ClN_3O_2$	GC
Triadimenol	Azole	$C_{14}H_{18}ClN_3O_2$	GC
Tricyclazole	Azole	$C_9H_7N_3S$	GC
Tridemorph	Morpholine	$C_{19}H_{39}NO$	GC
Triflumizole	Azole	$C_{15}H_{15}ClF_3N_3O$	HPLC
Vinclozolin	Phthalimide	$C_{12}H_9Cl_2NO_3$	GC
Zineb	Dithiocarbamate	$C_4H_6N_2S_4Zn$	HPLC
Ziram	Thiocarbamate	$C_6H_{12}N_2S_4Zn$	HPLC

Fungicides which appear in **bold** are those which are used most widely. (Reproduced from Jiménez JJ, Bernal JL, del Nozal MJ, Toribio L and Martín MT (1998) *Journal of Chromatogrphy A* 823: 381–387, with permission from Elsevier Science.)

detection limits and unambiguous spectral confirmation in complex matrixes.

Taking into account that the presence of heteroatoms is common, it is also possible to use the atomic emission detector (AED) to monitor characteristic wavelengths. Monitoring the emission lines for elements such as nitrogen, chlorine, phosphorus and sulfur ensures specific chromatograms for those elements, increasing the selectivity, which is especially desirable when dealing with environmental and food samples.

Multiresidue Methods

Multiresidue methods are desirable for the determination of specific components in samples of unknown origin or those which have been subjected to unknown pretreatments. Unfortunately, nitrogen-containing pesticides have been poorly investigated in comparison to the halogen- or phosphorus-containing pesticides as regards their possible combination in multiresidue methods. Nevertheless, there are several methods in which the behaviour of some fungicides is considered; some include up to 20 different fungicides. This type of research commonly relies on the use of more than one type of capillary column for the separation of broad groups of pesticides; usually the main column has a low polarity stationary phase, employing another one of mid or high polarity as a confirmatory column. The detection can be made directly or after derivatization, and using either a single or several detectors (ECD, NPD, FPD, AED, MS).

The use of the AED for multiresidue analysis partially overcomes some of the problems derived from poor resolution between compounds, as does GC–MS. However, many laboratories cannot afford GC–AED or GC–MS because they are more expensive than other options.

Practical Considerations

According to the aim, a technique will be selected, as mentioned before and this will determine the prior steps in the method. There are always some general recommendations, such as the need to employ standards and surrogates, whose addition (spiking) gives recoveries (which should be higher than 80%). The use of solvents of adequate purity is necessary; each batch must be tested for a potential source of interference; at the same time all glassware must be adequately cleaned. Apart from these general precautions, it is necessary to be aware of the importance of other aspects that have a notable influence in the analysis of fungicide residues. Some are summarized here.

Standards

One of the first and most important steps in fungicide residue evaluation in food and environmental samples is the correct preparation of standard solutions, preferably from solid reagents of certified purity, because of their low stability in solution. For example, Imazalil solutions are sensitive to light; Fosetyl residues decompose during storage at $-18^{\circ}C$. It is very common for derivatives to not last more than 24 h in a refrigerator; the stability should be checked for longer storage times. It has also been demonstrated

that the amount of fungicide residue in food is influenced by storage, handling and processing.

Sample Treatment

The sample may be simple or very complex; this will clearly have a great influence on the sample treatment. Isolation of the compounds using an extraction technique frequently needs a further clean-up step before determination. There are a great variety of possible approaches, from classic liquid–liquid extraction (LLE) to the use of supercritical fluids (SFE), and offline or online procedures.

Liquid–Liquid Extraction

There are many methods based on the use of a separating funnel, drying over anhydrous sodium sulfate and clean-up; Soxhlet extraction is also employed.

The commonly used solvents are ethyl acetate, acetonitrile, methanol, dichloromethane, acetone and *n*-hexane. Frequently, phase separation is hindered by emulsion formation in the separatory funnel, in which case filtration through a loose glass wool plug may be appropriate or another extraction procedure may be more suitable.

It is usual to find anomalous results for Vinclozolin, Captan, Folpet and Iprodione when LLE is used.

Solid-phase Extraction (SPE)

The laborious liquid–liquid partitioning clean-up procedures described in the literature have been replaced by fast SPE clean-up, with the additional advantage of a high enrichment factor.

The most recommended phase is octadecylsilane, although for some groups, the diol or cationic exchange phases may be better; graphitized carbon black is also a possibility nowadays. Florisil is frequently used to remove co-extractive interferences. When this is not sufficient, further clean-up can be achieved by gel permeation chromatography.

Solvent selection for recovery of fungicides from cartridges is very important. The results vary for individual compounds. **Figure 1** provides an example of the recovery of different fungicides and acricides from the analysis of must samples.

In all cases it is necessary to optimize the type of sorbent, sorbent mass, flow rate, sample volume, pH, ionic strength, drying time and soaking time. Sometimes the complete elution of the compounds from the disposable extraction column requires several portions of eluting mixture instead of only one. It is well known that, for conazole fungicides and Captan, low recoveries are obtained because the sample volume and flow rate of extraction seriously affect the recoveries.

In the analysis of modern fungicides, extraction with methanol, partitioning with chloroform, purification of the extract by column chromatography on sodium sulfate/Florisil/celite/charcoal is often recommended.

In the multiresidue methods acetonitrile is usually preferred, with SPE offline using C_{18} or polymeric cartridges, followed by GC–MS. This gives better

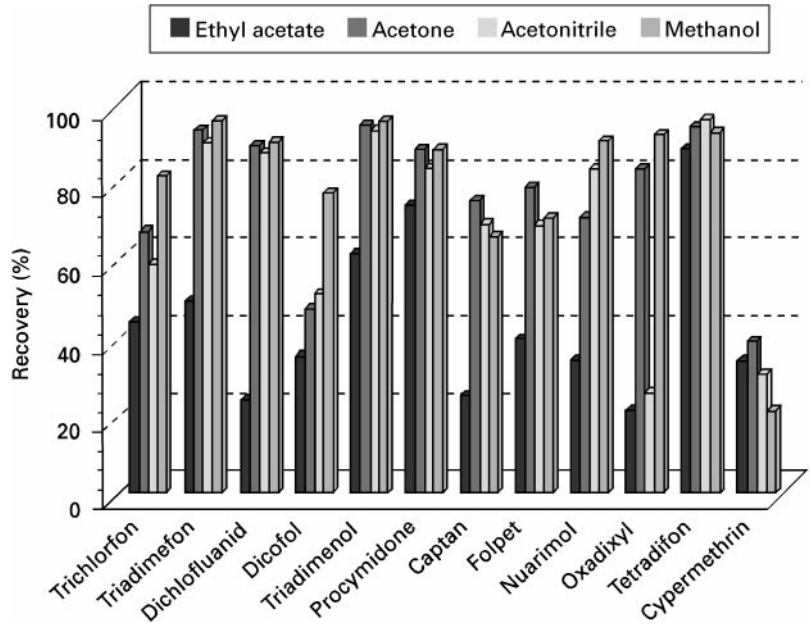

Figure 1 Recovery of pesticides from 500 mL of synthetic sample spiked with 4 μg L^{-1} by octadecylsilica cartridges eluted with 2 mL of solvent. (Reproduced from Bernal JL, del Nozal MJ, Jiménez JJ, and Rivera JM (1997) *Journal of Chromatogrphy A* 778: 111–117, with permission from Elsevier Science.)

results than online HPLC-diode array detection (DAD) which has drawbacks for trace level determination as a result of many interferences.

In water analysis, SPE on disc (C_{18} Empore) gives good results and it has been successfully applied to the determination of fungicide residues, but in Vinclozoline determination errors are obtained, with the major losses occurring when the fungicide was collected from the surface of the disc.

Solid-phase microextraction is also useful for the analysis of fungicide residues in water samples, although in complex matrices it gives low reproducibility, which suggests that it is only useful for semiquantitative purposes. In addition, the duration of the process in relation to other extraction procedures can seriously limit its application to large numbers of samples. The extraction conditions – stationary phase, time, temperature, type and concentration of compound and matrix – must be taken into account. In Vinclozolin and Captan residue analysis on semisolid spiked samples, lower recoveries are obtained when the amount added increases.

To prevent degradation or hydrolysis of certain fungicides (e.g. benzimidazoles), sometimes other extraction techniques such as those based on the use of pressurized hot water or supercritical CO_2 are recommended; even a cloud point preconcentration has been used, nevertheless, these procedures are not common as yet. The importance of sample preparation on the final chromatogram is seen from Figure 2; it can be observed that SPE gives the simplest chromatograms.

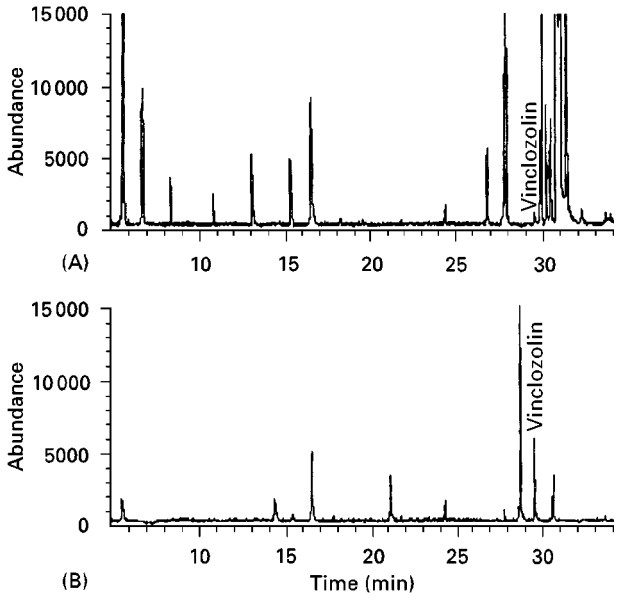

Figure 2 Chromatograms by EI-MSS (scan mode) for Vinclozolin residues in a larvae extract. (A) Hexane–acetone (70 : 30, v/v); (B) SPE. (Reproduced from Bernal JL, del Nozal MJ, Rivera JM, Jiménez JJ, and Atienza J (1996) *Journal of Chromatogrphy A* 754: 507–513 with permission from Elsevier Science.)

Matrix Effects

GC analysis for fungicides frequently presents considerable errors due to the so-called matrix effect which has been described in the analysis of diverse compounds in wine, grape juice, honey, milk, butter, fruits and vegetables. This effect is explained by a higher transference of analytes from the injection port to the chromatographic column either as a result of the presence in the extract of associated carrier substances from the matrix or of a protective effect in the injection port performed by these substances.

The matrix effect is usually greater for lower quantities of analyte, as can be seen in **Table 2**, where some data for common fungicides are shown. The recovery can also be influenced by the total amount of sample (**Table 3**). From both tables it can be deduced that serious errors can arise when the effect is not considered.

Once the sample preparation has been checked, attention must be paid to calibration. To reduce quantitative errors from the matrix effects, a standard addition method or an external standard calibration with standards dissolved in an unspiked sample extract can be used. The use of two or more certified reference materials to establish a calibration curve can help recognize matrix effects. If the measurement shows the same slope with the regression, it can be concluded that the matrix has no dominant influence.

In general, an analyte addition method (AAM), a sample variation AAM (varying the test sample mass and keeping the added analyte amount constant) or, better, a sample and analyte variation AAM must be used for calibration.

The Analysis of Various Fungicide Groups

Phthalimides

Captan, Captafol, Folpet There are several methods devoted to the analysis of residues of these compounds. Usually an extraction with *n*-hexane–acetone mixtures is carried out; after drying with sodium sulfate and evaporating the solvent, *n*-hexane is added. This is followed by clean-up on a Florisil cartridge, eluting it with an *n*-hexane–acetone mixture, evaporating and dissolving the residue in toluene and injecting an aliquot of the solution into the GC.

To prevent hydrolysis, cloud-point preconcentration employing the nonionic surfactant Triton X-114 has been proposed.

Table 2 Recovery (%) of some fungicides from honey samples, after conventional solvent extraction, spiked at different levels (average seven determinations)

Fungicide	0.025 mg kg^{-1}	0.125 mg kg^{-1}	0.25 mg kg^{-1}	1.0 mg kg^{-1}	2.5 mg kg^{-1}
Captan	1028	329	165	99	99
Folpet	2380	321	164	108	107
Iprodione	948	405	350	259	174
Vinclozolin	647	335	250	209	171

Captan and Captafol tend to decompose on columns that have been in use for some time. To avoid decomposition removal of the glass wool from the inlet of the GC column is recommended.

Benzimidazoles

Benomyl, Carbendazim, Thiabendazole, Thiophanate methyl This group is usually analysed by HPLC; nevertheless there are GC-MS methods using acid hydrolysis, re-extracting the amine and forming the *tert*-butyldimethylsilyil derivatives.

Carbendazim is a fungicide and the main metabolite for Benomyl, both of which are widely used on vegetables intended for direct human consumption. Methods usually provide residual levels in terms of Carbendazime because Benomyl degrades rapidly. To analyse Carbendazime by GC, the compound is usually extracted with ethyl acetate and derivatized with pentafluorobenzylbromide. After clean-up on a silica column, the product is determined by GC-ECD or GC-NPD.

Dicarboximides

Chlozolinate, Iprodione, Procymidone, Vinclozolin These compounds are frequently included in multiresidue methods and in many applications a significant influence of the spiking levels on recovery is observed. The most used techniques are GC-FID, GC-ECD and ion trap GC-MS in multiple ion-monitoring mode, with detection limits in the range of p.p.b.–p.p.t.

These fungicides are frequently investigated in wine analysis where it is known that the recovery not only depends on the concentration level but also on the variety of wine; attention must be paid to their metabolites, mainly those belonging to the 3,5-dichloroaniline group.

Table 3 Recovery of Vinclozolin from honey and larvae samples, spiked with 25 mg kg^{-1}, by solvent extraction with hexane–acetone at different proportions and octadecylsilica clean-up ($n = 5$) (Reproduced from Bernal JL, del Nozal MJ, Rivera JM, Jiménez JJ, and Atienza J (1996) *Journal of Chromatogrphy A* 754: 507–513, with permission from Elsevier Science.)

	Honey		*Larvae*
Sample amount	1 g	5 g	1 g
Extractant solvent (%)	Recovery		Recovery
Hexane (100)	45	42	48
Hexane–acetone (90 : 10, v/v)	64	63	67
Hexane–acetone (80 : 20, v/v)	81	82	78
Hexane–acetone (70 : 30, v/v)	99	98	95
Hexane–acetone (60 : 40, v/v)	99	98	96
Acetone (100)	99	98	95

Triazole

Bitertanol, Triadimefon, Triadimenol, Tryciclazole Usually they are analysed by GC-NPD and GC-MS in the selected ion monitoring mode. Triadimefon is easily reduced to Triadimenol, so both appear together.

Bitertanol and Triadimenol have diastereoisomers that cannot easily be separated; depending on their relative proportion they frequently produce peaks with shoulders.

Dithiocarbamate fungicides

These are habitually classified into three families of compounds depending upon their structure:

1. Dimethyldithiocarbamates (Ferbam, Ziram, Thiram)
2. Ethylenebisdithiocarbamates (Mancozeb, Maneb, Zineb, Nabam)
3. Propylenedithiocarbamates (Propineb)

It is very difficult to isolate and determine specifically the fungicides which belong to the same family due to the fact they possess the same organic moiety. Thus, the typical determination of these compounds is carried out as a group, performing an acid hydrolysis to carbon disulfide which is then quantified by techniques such as headspace GC-ECD.

When an FPD is used to detect CS_2, *n*-hexane must be avoided because it may co-elute, resulting in the quenching of the S emission in the detector.

EBDCs differ chemically from dithiocarbamates because they have reactive hydrogen on the nitrogen atom, which reduces their stability and results in different biological behaviour. One of the most characteristic decomposition products is ethylenethiourea (ETU). GC determination of ETU can only be achieved after derivatization, forming

trifluoroacetylated *S*-benzyl or butyl ETU derivatives that can be analysed by GC-NPD, GC-ECD or GC-MS. In real samples EBDCs and ETU content decrease with storage time. To prevent this, the addition of cysteine hydrochloride has been recommended.

See also: **II/Chromatography: Gas:** Detectors: Selective; Detectors: Mass Spectrometry. **Extraction:** Solid-Phase Extraction; Supercritical Fluid Extraction. **III/Pesticides:** Gas Chromatography. **Herbicides:** Gas Chromatography; Solid-Phase Extraction.

Further Reading

Barceló D (1993) *Environmental Analysis. Techinques, Applications and Quality Assurance*. Amsterdam: Elsevier.

Barceló D and Hennion MC (1997) *Trace Determination of Pesticides and their Degradation Products in Water*. Amsterdam: Elsevier.

Inspectorate for Health Protection (1996) *The Dutch Manual of Analytical Methods for Pesticide Residues in Foodstuffs*, 6th edn. Alkmaar, The Netherlands: Ministry of Public Health, Welfare and Sport.

Kidd H and James DR (eds) (1993) *The Agrochemicals Handbook*, 3rd edn. London: Royal Society of Chemistry.

Middleditch BS (1989) *Analytical Artifacts*. Amsterdam: Elsevier.

Milne GWA (1995) *CRC Handbook of Pesticides*. Boca Raton, FL: CRC Press.

Nielsen SS (1998) *Food Analysis*, 2nd edn. Gaithersburg, MA: Chapman and Hall.

Pleger K, Manner HH and Weber A (1992) *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*. Parts I, II and III. Weinheim: VCH.

Robinson J (1982) *Analysis of Pesticides in Water*. Vol. III. *Nitrogen-containing Pesticides*. Boca Ratón: CRC Press.

Thier HP and Kirchoff J (eds) (1992) *Manual of Pesticide Residue Analysis*, vols I and II. Weinheim: VCH.

Tomlin CDS (ed.) (1997) *The Pesticide Manual*, 11th edn. Farnhan, Surrey: British Crop Protection Council.

US Environmental Protection Agency (1990) *Methods for Determination of Organic Compounds in Drinking Water*. Springfield, VA: National Technical Information Service.

Liquid Chromatography

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Introduction

There are some groups of fungicides of wide use (benzimidazoles, ethylenebisdithiocarbamates) whose thermal instability, high polarity and low volatility make them difficult to determine by gas chromatography (GC) unless derivatization methods are employed. This usually makes the process longer and introduces new errors. These compounds are easily measured by high performance liquid chromatography (HPLC) as are many pesticides that were typically analysed by GC in the past. Integrated systems of solid-phase extraction sample cleanup and on line HPLC allows multiple options, not only by including fungicides of very different polarity in the same analysis but also by achieving very high concentration factors and, at the same time, analysing a large number of samples. The use of pre- or post-column derivatization reactions allows the analysis of compounds that are very difficult to determine or have a low sensitivity.

Given these advantages HPLC not only complements GC in fungicide residue analysis but is tending to displace it for many applications. Some considerations related to the use of HPLC are summarized below, with more attention being paid to the groups of fungicides most frequently determined by this technique.

Technique Selection

Most applications are based on the use of reversed-phase HPLC, nevertheless for some fungicides ion-pair HPLC (ethylenebisdithiocarbanates) (EBDC), micellar HPLC (Thiram) or chiral HPLC (Metalaxyl) are used. Normal phase HPLC, with amino-bonded stationary phases, is sometimes recommended, mainly for the benzimidazole group.

Chiral HPLC is very important for the determination of enantiomeric purity, mainly for large-scale synthesis. Resolution of C–chiral enantiomers seems to be easier than that of axial–chiral enantiomers (atropoisomers).

Columns

The most widely used stationary phases for fungicide residue analysis are the n-octyl and n-octadecylsilica because they allow the separation of compounds with a wide range of polarity. Some fungicides, mainly EBDCs, are easily ionized and because of this some