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IMPROVED COST-EFFECTIVE APPROACHES TO PESTICIDE RESIDUES ANALYSIS

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IMPROVED COST-EFFECTIVE APPROACHES TO PESTICIDE RESIDUES ANALYSIS

Sample collection and preparation stages are time consuming and labour intensive. However there are few obvious savings to be made in this area. It is necessary to collect an appropriately large sample and to reduce the sample size by well-tested procedures. The only important savings can be made by extracting a small final sample. Improvements can be made in the clean-up of the extract prior to final determination. This stage can be speeded up by reducing the sample size or by taking an aliquot of the extract through the clean-up procedure. Reduction of scale is possible with conventional equipment but, for true miniaturisation, specially designed laboratory equipment is needed. For many analyses, especially when methods are miniaturised, the use of internal standards (IS) can be valuable. The IS must behave in an identical way and be a compound of very similar chemical structure to the test compound. The IS should be added at the extraction stage and carried through each stage of the analysis. The usefulness of IS in pesticide residue analysis has become accepted and their use should be encouraged. Recovery data for the IS and for the compound to be determined, as well as relative response factors, should be published together with the results. In recent years, a variety of laboratory procedures have been automated. Fully automated GLC and HPLC procedures are available for the determination of pesticide residues at the subnanogram level. The latest developments in automation and miniaturization of the extraction and the clean-up have shown that this difficult and costly part of the analysis can be automated and hopefully integrated with the well-developed step of determination by gas and liquid chromatography. A more radical alternative to improving residue analysis is by adopting immunochemical techniques. Immunochemical techniques especially the ELISA method offers great potential and exploration of this area by residue chemists should be encouraged.

1. INTRODUCTION

The presence of pesticide residues in food, wildlife and the environment is of growing concern. To meet modern pesticide regulatory requirements there is a growing demand for data on the metabolism and degradation of pesticides and for monitoring of residues in food and in environmental samples.

The costs of carrying out residue studies are increasing rapidly and therefore there is a need for simplified and cheaper analytical procedures.

Over the past two decades, many advances have been made in residue analysis with the application to gas chromatography linked with specific detectors (including the mass spectrometer) and, more recently, by extensive use of high performance liquid chromatography. However with the increasing cost of manpower, solvents, equipment and laboratory space, there is an urgent need for residue chemists to become more aware of developing cost-effective procedures.

There are a number of approaches to reducing costs. The most suitable approach will vary from laboratory to laboratory depending on local circumstances. For instance, where costs of, and difficulties with, sophisticated equipment are more limiting than manpower costs, the use of simplified procedures will be essential. The IUPAC Commission on Pesticide Chemistry has recently reviewed these procedures (Ref 1). Where manpower costs are the limiting factor, sophisticated methods and automation of methods will be most attractive.

Costs would be reduced if some stages of residue analysis could be shortened or eliminated. Chromatographic clean-up procedures have been widely used in residue chemistry and residue analysts have become highly skilled in their use. However alternative approaches such as immunological methods, which would eliminate extensive clean-up of samples, are attractive.

To become more cost-effective in generating data, residue analysts will be required to adopt newer techniques and approaches. At the same time, the limits of sensitivity and the accuracy of measurement must be retained.

This report indicates some of the changes which are taking place in residue analysis and highlights areas where alternative approaches need to be explored further. The information should help analysts to become more efficient and should encourage the introduction of newer methods to generate residue data.

2. APPROACHES TO SAMPLING

2.1 Introduction

The collection and subsequent preparation of a residue analytical sample is a vital but laborious and time consuming operation. However, it is important that both these stages are carried out carefully and in accordance with an agreed protocol. Approaches to doing these procedures well and as cheaply as possible are discussed.

2.2 Field Sampling

The objective of residue analysis is to obtain a reliable measurement from which the residue present on a crop, in a soil or in other types of sample can be determined. Clearly, however well-conducted the analysis is, the result will finally depend on how representative the sample was of the lot under consideration.

There is no absolutely ideal way to collect the primary samples. It is important that the analyst thinks carefully about his specific aim and the nature of the particular trial. Only then can an appropriate sampling procedure be chosen.

To ensure that the sample is representative, it is essential to take a sufficient number of individual items from the lot. There are a number of approaches to taking these primary samples. One suggested approach is to select at random eight samples on a random number basis (Ref 2). These samples are then mixed in 2 groups of 4 samples each and the two basic sub-samples, sub-divided in two steps to give eight analytical samples.

This random approach has some appeal but carried through fully has the obvious disadvantage of requiring eight separate analyses to provide a final residue level. The approach is valuable where a very accurate answer with a measure of precision is needed. It is however costly in analysis time. A second method is to use a systematic approach with some random elements. An example of this is to collect a number of primary samples along an imaginary W drawn on the plot. The primary samples are collected at intervals along the four arms of the W. Approaches of this type are often used in practice.

The numbers of items collected will depend on a range of factors eg, the size of the trial, the size of the items and the accuracy desired in the final result. Inevitably some compromises have to be made.

Most pesticide companies have residue sampling guides which recommend the number of items or the total weight which should be collected. Much of the above has been reviewed (Ref 3) and have recently become incorporated into internationally recognised sampling guidelines (Ref 5).

Not all the recommendations in these documents are based on proven experimental data. Much is based on traditional approaches to sampling and a general commonsense view. However, to obtain a field sample which is representative of a whole plot, it is generally accepted that the recommended approaches should be followed.

Although the recommended methods may be time consuming, short cuts at this stage are likely to lead to non-representative sampling and hence invalidate all the analytical data finally generated.

Soil sampling warrants special mention as it is particularly difficult to obtain a meaningful, representative sample especially from an 'in-use' study where a crop is growing on the plot. Again it is important to take a sufficient number of core samples (usually 20-50) from different parts of the plot.

One needs to sample to an agreed depth and the core can then be sectioned, if needed, into various levels to measure the distribution of a chemical down the soil profile. It has been shown (Ref 6) that the diameter of the soil cores makes little difference to the analytical result.

Examples of problems encountered in soil sampling are:-

1 Soil too dry This makes the corer hard to insert and the core may break up as it is removed.

2 Soil too stony This again makes the corer difficult to insert and it can disrupt core profiles by causing compaction. A number of stones in a core can also affect the analytical result.

3 Plot cultivation When sampling a ploughed or a ridged field, it is difficult to decide soil depths. Cultivation can also affect the distribution of the chemical down the soil profile and may move the chemical out of the sampling zone.

Recently a "zero contamination sampler" has become available.* This has overcome some of the problems of soil sampling but this still remains a difficult subject. To overcome some of these problems, it is necessary to choose soils which are not too stony and to reject cores which are incomplete. The samples need to be taken with care and attention.

2.3 Reduction in Size of Field Samples

The amount of the field sample is normally too large for analysis and has to be reduced in size to an appropriate amount prior to analysis. During this stage, it is vital that:-

a) no contamination takes place and no degradation of residue or deterioration of the sample occurs.

b) the sample size is reduced in a way which retains the representative nature of the field sample.

This process should normally be carried out at the analytical laboratory rather than in the field. However in the case of certain supervised trials where an experienced person, with special training in residue sampling is used, some size reduction in the field is acceptable. It is imperative that the trained person is aware of the objectives of the trial, is scrupulously careful to avoid any cross-contamination of the samples and ensures that no degradation of the residue will occur.

At this stage, the sample size can sometimes be reduced by half by quartering large items and returning 2 opposite quarters to the laboratory. Quartering should be done down the vertical axes of the crop unit. It is possible to take small samples from individual samples by techniques such as the use of cork borer. However this will not necessarily produce a representative sub-sample and without supporting data, such techniques must be used with great caution.

Whether this process takes place in the field or in the laboratory, further size reduction is necessary prior to analysis. Normally the remaining sample is chopped or ground in an appropriate machine so that a well-mixed, sub-divided sample is obtained. A range of commercial choppers, mincers and grinders are suitable.

The 'Analytical Sample' is taken from this homogenised sample. Normally 20-50g of sample is used for the analysis. This size is probably required for many analyses especially when methods have only recently been developed and when the limit of determination dictates this sample size. However when the method has been well-tried and the homogeneity of the sample ensured, the use of smaller sample size can often lead to economies in the use of solvent and in speeding up the method.

Provided that the laboratory sample is chosen from a properly homogenised sample, it is possible to obtain accurate analytical results from very small samples especially if analytical precision is improved by the use of an internal standard method. The data in Tables 1A and 1B illustrate this (Ref 7).

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* Available from Clements Associates Inc., P O Box 398, Grinnel Iowa USA.

TABLE 1 Replicate analysis on various sample weights of cabbage and apples containing cypermethrin residues

(Samples were prepared according to a standard, thorough chopping/mincing process).

TABLE A Cypermethrin residues (mg kg^{-1}) determined using ICI Plant Protection Division Residue Analytical Method No.56 - ie internal standard methodology

Crop \ Sample weight	50g	25g	10g	5g	2g
Cabbage	0.399 0.385 0.412 0.392 0.413	0.405 0.452 0.445 0.380 0.405	0.389 0.408 0.421 0.400 0.400	0.445 0.467 0.503 0.412 0.448	0.417 0.469 0.422 0.483 0.543
Mean rel.sd	0.400 3.1%	0.420 7.0%	0.404 2.9%	0.455 7.3%	0.467 11%
Apple	0.255 0.244 0.231 0.224 0.268	0.239 0.205 0.177 0.253 0.251	0.241 0.241 0.200 0.246 0.253	0.221 0.204 0.386 0.282 0.258	0.194 0.310 0.217 0.215 0.160
Mean rel.sd	0.244 7.3%	0.225 15%	0.236 8.8%	0.270 26%	0.219 25%

TABLE B Cypermethrin residues (mg kg^{-1}) determined using ICI Plant Protection Division Residue Analytical Method No.42 - ie external standard methodology

Crop \ Sample weight	50g	25g	10g	5g	2g
Cabbage	0.401 0.401 0.411 0.436 0.406	0.552 0.508 0.482 0.522 0.431	0.408 0.459 0.449 0.305 0.352	0.479 0.469 0.548 0.406 0.532	0.412 0.426 0.424 0.492 0.510
Mean rel.sd	0.411 3.5%	0.499 9.1%	0.395 17%	0.487 12%	0.453 9.9%
Apple	0.287 0.269 0.269 0.242 0.305	0.295 0.224 0.207 0.281 0.293	0.270 0.231 0.210 0.306 0.284	0.238 0.216 0.388 0.282 0.283	0.206 0.299 0.218 0.237 0.151
Mean rel.sd	0.274 8.5%	0.260 16%	0.260 15%	0.281 24%	0.222 24%

With cabbages, using the internal standard method, the result was similar for sample weights ranging from 50g down to 2g. The relative standard deviation for repeat analysis at each sample weight was acceptable. A similar picture emerged using the external standard method except that the relative standard deviations were somewhat more variable.

Using apples, where the residue is on the skins and the skins are more difficult to homogenise, again similar results were obtained at all sample weights between 50g and 2g using both internal and external standard methods. However at the 2g and 5g weights, the standard deviations of the results indicated significantly poorer precision.

It appears probable that considerable savings in solvents and time can therefore be achieved by careful use of smaller sample weights. It would be desirable however to see more data to illustrate this.

3. MINIATURISATION OF THE CLEAN-UP STAGE

3.1 Introduction

Until recently, during the development of extraction and clean-up procedures for pesticide residues, aspects of economy were not given prime consideration. The increasing requirements for residue data, however, emphasise the need to look for approaches to carrying out the extraction and clean-up stage of pesticide analyses more rapidly and effectively. One approach is the use of miniaturized methods which can save cost and time for the individual residue determination.

A critical review of the methods currently available shows that nearly all of them start from an analytical sample which is much larger than needed for the subsequent determination step. Thus, in the majority of clean-up procedures prior to a determination by GLC or HPLC, 50 - 100g of plant tissue or soil or 0.5-1l of water have to be extracted. Consequently both extraction and clean-up steps require large volumes (300 - 600ml or even more) of solvents which must be highly purified and are therefore very expensive. At the same time, a lot of large size glassware, extensive space and above all much time and manpower are needed within the laboratory for handling the large solvent volumes and for concentrating them again. GLC and HPLC, however, are so sensitive that only a minor portion of the purified extract is required for the determination: while the final solution is usually adjusted to 1-5ml, only 1-5 µl need to be injected for GLC or 50-100 µl for HPLC. The remaining portion may be useful for confirmation purposes but, in most cases, this is not done and the excess is rejected. It is not economic to proceed in this way.

The best way to improve this would be to miniaturize the clean-up methods, so that all analytical parameters such as sample size, quantity of solvents and adsorbents, volume of glassware etc. are reduced, say by a factor of 5 or 10. If this approach is taken for all stages of the clean-up, some problems are raised. These are considered below.

3.2 Some Factors Restricting Miniaturisation in Analytical Practice

The equipment and facilities available in the standard pesticide residue laboratory permit miniaturisation to only a limited extent. For instance, the usual glassware (separation funnels, evaporation flasks etc) is so designed that volumes down to 2 - 5ml can still be handled by a skilled analyst, but for volumes of less than 1ml, it is no longer suitable. True miniaturisation therefore requires special equipment and more sophisticated tools such as those applied in the field of organic microchemistry. This aspect, however, is covered separately in section 3.3 and the discussion here is restricted to the possibilities offered by normal laboratory facilities.

The sample size is also a limiting factor for miniaturisation. There are only few substrates in which pesticide residues are homogeneously distributed, eg. the non-polar organochlorines in fats or polar organophosphates in water. A uniform distribution of residues can never be assumed in plant tissue or soil samples. A small sample aliquot may therefore not be representative and might not reproduce the average residue concentration of a greater lot, eg, of plant material grown in the field or taken from the market.

An additional restriction is that some pesticides are chemically altered or even destroyed (eg, dithiocarbamate fungicides) when plant tissue is chopped or minced in order to obtain a small homogeneous sample aliquot. However, by careful and thorough sample preparation, a small but representative sample can be obtained. This can sometimes be achieved by adding dry ice or sodium sulphate at the grinding stage. The stability of a residue in the sample should always be checked.

Finally to obtain an acceptable limit of determination special equipment must be used to adjust the volume of the purified extract solution to a definite volume of less than 1ml.

In principle, this problem could be easily overcome by evaluating the chromatograms by an internal standard. This would make it possible for the final solution to be concentrated to a volume of 1 or 2 drops which need not be exactly defined. The use of an internal standard, however, has not been commonly accepted in residue analysis, particularly not for multiresidue procedures. The main argument is that unknown co-extractives could change the size of the standard peak and cause false results. However by careful choice of internal standard, this can be avoided. As an extra precaution, two internal standard substances can be used. The constant relation of their peak areas allows the absence of interferences to be checked. The use of internal standards allows all recoveries to be checked and their use in residue analysis should be encouraged (see Section 4).

In most methods, the unfavourable ratio of concentrated to injected volume has not been varied. Obviously the inferior detection limit in these cases is considered acceptable compared to the saving of cost and time. This is particularly true when screening analyses are carried out for regulatory purposes by GLC with the highly sensitive ECD. Moreover, this problem can be overcome to a certain extent by using capillary columns for the GLC separation: the improved peak shape enhances the detectability, especially when a splitless injection technique is applied.

3.3 Examples for Successful Miniaturisation

Complete Clean-up Method

Despite some of the problems discussed above, numerous laboratories have already gained useful experience with analytical procedures in which the size of the entire clean-up had been reduced. Certainly it has been for reasons of sample homogeneity that such methods have so far been applied almost exclusively for the clean-up of fats, oils and the lipid fraction extracted from foods with high fat content. Since no extraction step is required here, it is a simple matter to reduce the size of the usual adsorption column clean-up for the analysis of organochlorines. The amount of fat, adsorbent and eluting solvent is mostly smaller by a factor of 5 or 10 than in the corresponding "macro" methods, eg, 100mg fat, 3g Florisil (3% water), 30ml petroleum ether/ dichloromethane 8 + 2 (Ref 8); 100mg fat, 4g silica gel (30% water), 50ml petroleum ether (Ref 9); 40-50mg fat, 2g basic alumina (10% water), 15ml hexane (Ref 10).

Such methods, which save time and cost, are particularly well suited for screening analyses where many samples have to be analyzed. As the volume of the final solution, however, is not reduced and the solution is concentrated as usual to 1ml, the determination limit is correspondingly poorer. Additionally, special care has to be given to the purity of solvents, adsorbents etc as already small interfering peaks can more easily give rise to misinterpretations.

The greatest progress in miniaturising residue methods to date has been made by Rado and Gorbach (Ref 11) who have applied the principles of microchemistry to their methods. Special equipment has been designed, transfer techniques have been modified and even the typical laboratory work place has been redesigned so that most operations can be carried out from a sitting position. The methods they describe should be applicable in many laboratories to a wide range of residue methods. Applications of their techniques will afford savings in solvent use and in operator time.

Individual Clean-up Steps

It is difficult in some cases to miniaturise the complete analytical procedure. In many cases, however, improvements in costs can be made when merely the size of some individual clean-up steps is reduced.

A first approach is to use only a minor aliquot of the crude extract for further clean-up. This has already been done in some methods for plant material but the resulting reduction of the consecutive steps has usually been rather small and cannot be considered a true miniaturisation.

a) Gel Permeation Chromatography

Reducing the column size in GPC should not raise any difficulties, for the separation would remain just as effective if column length is kept constant but column diameter and sample loading are reduced. However, only a few attempts with this have been made (eg, Ref 12), and there are no systematic reports about the possible extent of miniaturisation in residue analysis.

b) Adsorption Chromatography

Adsorption columns are most effective and are, therefore, most readily miniaturised if the residue in question and the co-extractives to be removed differ greatly in their polarity. Nearly ideal conditions are found in some derivatisation reactions which allow the derivative formed to be quantitatively separated from the excess reagent by means of a very small column of silica gel, alumina etc.

In the clean-up of sample extracts, however, great differences in polarity are very seldom encountered and the excellent separation of organochlorines from fats which had been cited above is unfortunately unusual. It can be expected, therefore, that miniaturisation of adsorption columns will not be very promising for efficient removal of co-extractives. This is particularly true for multiresidue procedures, and it is not possible to name any important method in which interfering materials are removed with a mini-adsorption column. Nevertheless, the size of silica gel, alumina, and Florisil columns, proposed in the literature in recent years, is becoming smaller.

Additionally, some interesting applications of small columns have been reported. For instance, a 1g silica gel column is used within the scope of a broad-spectrum multiresidue procedure following a normal size GPC clean-up step. It gives additional purification of non-polar fractions by removing co-extractives interfering with EC detection. For those residues which are eluted with more polar solvents and are analyzed with the less sensitive FPD or TID, thus not requiring the purification, the elution behaviour gives additional information about the identity of a residue (Ref 13).

c) Disposable columns and cartridges

The preparation of mini-adsorption columns requires almost the same time as for columns of greater size. The developement of small disposable columns or cartridges therefore facilitates progress in this field. One form which is commercially available (Sep-Pak, Waters Associates) consists of polypropylene capsules filled with silica gel, C-18 reversed phase material or similar packings of preparative LC quality.

The use of such cartridges enables subsequent steps to be simplified. The main field of application will be the concentration of residues from a dilute solution on the Sep-Pak material and the subsequent elution in a small volume of another appropriate solvent (eg, Refs 14,15). In some cases, these materials are also useful for some clean-up when a crude extract solution or sample homogenate is forced through the cartridge (eg, Ref 16).

The use of the cartridges is, however, limited by the same restrictions which have already been discussed for mini-adsorption columns. As the separation depends on differences in polarity which are usually not very distinct, there will be only few fields where cartridges can be effectively applied, to multiresidue analyses. This general drawback is also reflected by the fact that hitherto only few publications have reported the successful use of this new tool in pesticide residue analysis. However their use is often valuable in the analysis of single compounds. Useful examples are given in Ref 117.

4 USE OF INTERNAL STANDARDS

4.1 Introduction

The use of internal standards (IS) in pesticide residue analysis is still relatively rare compared to their use in pesticide formulation and drug analysis. Nevertheless, an increase in their use can be noted, probably caused by several factors, such as:

- improvement of gas chromatographic technique (better columns, detectors and auxiliary apparatus give better chromatograms, leaving space for the extra peaks of internal standards);
- increased awareness of time and costs of residue analysis (especially when long series of samples must be analysed, the use of internal standards saves time);
- introduction of automated data handling in residue laboratories (calibration and calculation is more easily accomplished by such systems with internal than with external standards).

As discussed in the previous section, there are problems with the use of internal standards in pesticide residue analysis. However, if these are carefully guarded against, many advantages can be gained from the use of procedural internal standards, ie those which are added at the primary extraction stage and taken through the entire analytical method.

4.2 Choice of Internal Standards

For residue analysis, it is important to choose 'true' internal standards, ie, those which behave in an identical manner to the analyte in all significant physical and chemical characteristics. Usually only very close analogues fit this description. The use of stable isotope labelled IS in gas chromatography - mass spectrometry ion monitoring assays is practically ideal but obviously not applicable to more conventional GLC and HPLC methods. Radiolabelled forms of the analyte added in amounts below the limit of detection by GLC or HPLC methodology can be used to monitor the analytical recovery in each sample provided the specific activity of the radiolabelled form is high enough to allow accurate counting at low absolute chemical levels. The extra costs involved with the counting procedure however can off set the general IS cost savings. More typically close analogue of the analyte, eg a methyl substituted form, can be used especially when the substitution is in an 'innocuous' position in the molecule thereby not significantly altering the chemistry of the compound.

When internal standardisation is used simply to control the volume of the final sample solution or that which is injected into the analytical instrument then 'true' internal standards are not required. The IS in this case must simply chromatograph in a convenient manner.

4.3 Factors Governing Choice of an Internal Standard

Certain parameters must be observed for a 'true' procedural internal standardisation technique. Commonly, at the onset of method development, calibration lines should be obtained from a sample containing a set amount of internal standard and varying amounts of analyte. The 'extracted' calibration line obtained for the response ratio of analyte to standard plotted against the concentration of the analyte, should be examined for:

a. linearity : usually by means of regression analysis
and b. whether or not the line passes through the origin (within practical limits).

Given that a linear calibration curve which passes through the origin is obtained then a basis for a true IS method is achieved. Such methods can be calibrated using 'single point ratio' standards but it should be noted that the standard should be added at the extraction stage in the method. Should further examination of the method show that the slope of the calibration line for the standard added at the extraction stage is identical to the calibration line for the pure standard (non-extracted) then it is not necessary to run an extracted standard. This allows further savings.

Internal standardisation can obviously still be used even when the calibration line is not linear and does not pass through zero but, in these cases, extracted calibration curves must be run with each batch of samples and hence many of the advantages in terms of cost saving are lost.

Although it is not absolutely necessary for high "% recovery" values to be obtained for analyte and standard during IS methods, this is still very desirable on the grounds of analytical confidence, overall detectability and 'robustness' of the method. This latter point is similarly enhanced if the extracted curve and the non-extracted line the have same slope ie both standard and analyte have identical % recovery which is independent of the concentration of the analyte.

When one decides to use an internal standard method, it should be noted that poor internal standards are useless and that invalid assumptions regarding linearity etc etc must always be guarded against.

The discussion above is obviously most relevant to the case where only one analyte is measured. Multiresidue methods are correspondingly more difficult to calibrate in this way and hence require much more effort in method development. It is possible however to have, for example, pairs of internal standards which bracket a certain class of pesticides which elute in a specific fraction from an adsorption column.

Some regulatory authorities, prefer that internal standards are not used, mainly because they are not entirely compatible with analysing samples of unknown history with no 'untreated control' as a reference point. However, this does not mean that IS methods cannot be employed in industrial residue chemistry laboratories with all the subsequent cost saving. Conventional external standardisation methods can be supplied as "Regulatory methods".

4.4 Advantages to be gained from the use of internal standards

i. Improved precision : Typical relative standard deviations obtained for repeated assay of the same sample can be <5%. Unlike external standardisation (ES) this precision is not adversely effected by a reduction in solvent volumes, thereby allowing extensive miniaturisation of procedures.

   Similarly, the improved precision is independent of absolute recovery through the method and hence lower efficiencies for individual steps, eg liquid-liquid partition, can be tolerated. For solvent partition, often a single pass is sufficient for internal standardisation (IS) whilst three might typically be required for methods using external standards.

   A further point arising from the improved precision obtainable with IS methods is that they are not so critically dependent upon the skills of the residue analyst and, in general less experienced workers can achieve excellent results with IS methods.

ii. Improved analytical confidence : As each sample effectively acts as its own recovery check, analytical failures are immediately obvious. This can preclude the need to run duplicates on each sample with a subsequent reduction of resources reduction of resources and costs. Once the internal standard method has been validated for a particular application then there is not need to run further check samples other than an untreated control. Typically ES analytical runs may comprise 10 samples plus 2 recoveries and 1 control. By substituting the recovery samples for additional test samples, significant savings can be made when large numbers of samples are analysed.

iii. Improved derivatisation methodology : The use of derivatisation reactions in residue analytical chemistry can suffer greatly from the effect the presence of the substrate has on the yield of the reaction. True internal standards, which have identical functional groups to those of the test substance can be used to overcome this potential source of error. It is important to ensure that the IS and the analyte react consistently with the derivatising agent even in the presence of sample extracts.

4.5 Survey of Literature

Literature from 1977 onwards was screened for references to the use of internal standards in pesticide residue analysis.

For convenience, the references are grouped in two tables: Table 2 summarises the methods using GLC, Table 3 those using HPLC. Not published, but known by personal communication, is the use of telodrin, methoxychlor, 1,2,3,4,5-pentachloro-biphenyl or

TABLE 2 Papers describing the use of internal standards in pesticide residue analysis by GLC

Pesticide	Matrix	Internal Standard	Ref
biphenyl and metabolites	microbial extracts	naphthalene, resp. 1-naphthol	17
biphenyl, o-phenylphenol	citrus	anthracene	18
carbophenothion	goose tissues	chlorfenvinphos	19
2,4-D, 2,4-DB	water	2,4,5-T	20,21
2,4,-D, 2,4-DP-TP 2,4,5-T, 2,4,5-TP	water, soil	2,3,4-T	22
o,p'-DDD	urine, blood	m,p'-DDD	23
o,p'-DDD	plasma	p,p'-DDD	24
o,p'-DDD	plasma	p,p'-DDD	25
DDT	quail eggs	aldrin	26
dichlofluanid	must	dieldrin	27
dimethoate	wheat	methylstearate	28
endosulfan	urine, serum tissue	D-labelled endosulfan	29
ethoxyquin	apple	tetrahydroquinone	30
hymexazol	rice, -straw	diazinon	31
isoxathion	crops, soil	triphenylphosphate or cyanofenphos	32
MCPA	soil	2,6-dimethoxyphenol	33
niclosamide	water	5-dechloro-niclosamide	34
paraquat	blood	diethyl-analogue	35
paraquat	body fluids	diethyl-analogue	36,37
paraquat	plasma	diethyl-analogue	38
PCB's and organochlorine pesticides	fish	octachloro-biphenyl	39
pentachlorophenol	rainbow trout	pentachlorophenetole	40
pirimiphos-ethyl	vegetables	diazinon	41
procymidone	grape, must, wine	4,4'-dichlorobiphenyl	42
2,3,7,8-TCDD	fish	^{14}C-TCDD	43
2,3,7,8-TCDD	fat, milk	^{17}Cl-TCDD	44
trifluralin	water, soil	lindane	45
triforine	vegetables	1,2-dibromoethane	46

TABLE 3 Papers describing the use of internal standards in pesticide residue analysis by HPLC

Pesticide	Matrix	Internal Standard	Ref
azinphos-methyl	rat liver	benzamide	47
bendiocarb	wool	methylbenzoate	48
carbaryl	water, milk, vegetables	pentachlorophenol	49
difenacoum, brodifacoum	bait	1,3,5-triphenyl-benzene	50
paraquat	sunflower seed	diethyl-analogue	51
vinclozolin	grape	benzene	52
warfarin	plasma	methyl-warfarin	53

(±)heptachor-epoxide (the non-natural isomer) as internal standard in the analysis of organochlorine pesticides by several laboratories in the Netherlands.

4.6 Discussion of Literature Survey

4.6.1 Choice of the Internal Standard

The above survey is not fully exhaustive but it allows general observations to be made. From Table 2 and 3 it can be seen that very different types of pesticides have been analysed by internal standard methods, but that there is little consistency in the choice of the internal standard. Some choices seem logical, such as naphthalene or anthracene for biphenyl, DDT for DDD, 2,4,5-T or 2,3,4-T for phenoxyacetic acids or the diethyl-analogue of paraquat for paraquat. Other choices however seem less logical such as methylstearate for dimethoate, dieldrin for dichlofluanid (here the two peaks were moreover only partly resolved) or lindane for trifluralin. Only two references (Refs 22,39) mention the use of an internal standard in a multiresidue method (viz. for organochlorine pesticides and for phenoxyacetic acids respectively). Application of internal standard methods to organophosphorus pesticide residues (potentially a wide field of interest) seems little explored.

4.6.2 Recovery of Internal Standards

Few papers mention the actual recovery of the internal standards used. This can be justified if the internal standard is added together with the extraction solvent and no further clean-up or derivatisation is needed. If the extract is to be processed, however, the recovery of the internal standard might be different from the recovery of the pesticide to be determined, and this may lead to erroneous results. Thus, when the procedure described by Winell (Ref 30) for the determination of ethoxyquin (where tetrahydroquinone is used as an internal standard) was applied, 100% recovery for the internal standard, but only 60% recovery for ethoxyquin was found. Corrections for differences in recovery are possible, but, as lower recoveries tend to be less reproducible, this adversely affects the reliability of the result. This also holds when the recovery for both internal standard and compound to be determined is low.

4.6.3 Determination of Relative Response Factors

Determination of relative response factors is an essential point in applying internal standard methods. It can be most useful if those factors are in fact published together with the results obtained, so that the reader can get an impression as to how far the factors deviate from ideality.

5. AUTOMATION OF RESIDUE METHODS

5.1 Introduction

In recent years, increasing amounts of effort have been devoted to the development of partial or fully automated procedures. Several reviews on automation in pesticide residue laboratories provide information on the latest developments in sampling, extraction, cleanup and determination of residues in different matrices (Refs 54,55,56). Automation is likely to be particularly valuable when combined with miniaturisation.

Recent developments in GLC and HPLC technology have provided the basis for miniaturisation and automation of the analytical procedures. These changes included employment of highly sensitive and specific detectors, improvements in column technology, automated injectors and on-line computers to control the operation and for data processing. Less progress toward automation was made in the sampling, extraction and clean-up procedures, which remained the more difficult and costly part of the analysis. Automation has its advantages and drawbacks, but everything points towards more automation even if instrumentation becomes very expensive. This will be especially true in laboratories where many similar analyses are performed.

5.2 Extraction

Pesticide residues are extracted from the sample matrix by suitable solvents and the solid particles are removed by filtration or centrifugation. Several approaches to automation of these steps were reported. The preparation of soil samples has been

automated by Marsh (Ref 57). The samples were automatically prepared, weighed, extracted and transferred to an autoanalyzer. An automated sequential sampler for GLC has been described for trace airborne pesticides (Ref 58). A purge-and-trap concentrator for liquids and gases was developed by Bellar and Lichtenberg (Ref 59). Volatiles were purged by an inert gas and trapped in a resin-filled tube. The sample was desorbed by heating the trap and transfer of the sample to a GLC column. Such instruments are produced commercially by: Chemical Data System, NuTech Co., Spex Ind., Tekmar, and Hewlett-Packard. A simple liquid-liquid extraction which produces a counter-current type extraction has been described by Van Tooren (Ref 60).

Automation of the extraction of plants was described recently (Ref 61). The extraction was carried out with a commercially available module called the SOLID prep II Sampler made by Technicon Inst. Co. The sampler consists of a turntable with polypropylene sample cups, a metering pump for dispensing solvents into the homogenizer, connection to vacuum for draining out excess sample or solvent, and an optical programmer for controlling the functions. The solvents used in this module were acetonitrile and chloroform. The SOLID prep Sampler has been modified to accommodate a larger solid sample (40g soil sample Ref 62).

5.3 Clean-up

Most extracts are not clean enough for direct analysis and require clean-up to remove interfering substances. The clean-up is usually achieved by liquid-liquid partitioning and/or by column chromatography and TLC. Several procedures were described for the automation of the clean-up. An apparatus, for rapid extraction of small volumes of one liquid phase with another, immiscible one was described by Beroza (Ref 63). An automated spotter that was coupled with an optical scanning head, provided an automated device for clean-up and determination of pesticide residues (Refs 64 and 65).

The introduction of gel permeation chromatography (GPC) enabled the clean-up of a wider range of pesticides, including the more polar pesticides and their metabolites. Since macro-porous particles are suitable for recycling, the column packing can be regenerated and therefore this technique could more easily be adapted for automation (Ref 66). The column packings that have been used were prepared from carbonaceous resin: Amberlite XE-340 (Ref 61) Bio-Beads SX-3 (Ref 67) or XAD-2 (Ref 68).

In order to integrate the various steps into a fully automated procedure, it is necessary to automate the filtration, decantation and concentration of the extract. Getz et al. described an approach to do this. A multichannel peristaltic pump in the SOLID prep II Sampler was used to pump out the sample onto a continuous filter, a roll of Whatman filter paper being drawn continuously over a Teflon platen. The filtered sample was debubbled to remove the air before arriving at the head of the clean-up column (Ref 61).

A procedure for solvent concentration by evaporation was described by NASA (Ref 69). The sample flows downward in the coil into an inert gas that is flowing upward. Another device from Technicon, the Evaporation-to-Dryness Module (EDM), has also been described (Ref 56). The sample in one solvent was taken to dryness and redissolved in a smaller volume of a second solvent.

Sample concentration was also achieved by adsorption of the residues on to an appropriate matrix. The Sep-Pak cartridges from Waters Associates or other adsorbents, such as Porapak, activated carbon, etc., are useful for this purpose. Some degree of automation was obtained by employing the DuPont Prep I Automated Sample Processor. Distillation as a clean-up or concentration step in the automated program has been examined (Ref 70).

Sweep co-distillation, the Storherr approach to the clean-up of pesticide residues, was further developed and automated by Dingle (Ref 71). The method was employed successfully for the clean-up of organophosphorus and chlorinated pesticide residues in many crops (Refs 72 and 73).

5.4 Pesticide Residue Determination

Several non-chromatographic automated procedures were developed for the analysis of pesticide residues. Automated colorimetric and anti-cholinesterase methods were described for organophosphorus and carbamate pesticides (Refs 74, 75, 76, 77, 78). Autoanalyzers are used in some of these procedures.

An automatic spotting device for TLC was developed and combined with an optical scanning detector for the quantitation of the resolved spots (Ref 65).

The development of highly efficient automatic injection devices for GLC and HPLC and the aid of computers to control the operation and to process the data made the analysis by chromatography a fully automated process. Very specific and sensitive detectors for GLC enable the detection of picogram quantities of halogen-,phosphorus-,nitrogen- and sulfur-containing pesticides. Gas chromatography - mass spectrometry achieved sensitivity permitting complete spectral analysis in the nanogram range and for selected ion monitoring even in the picogram range.

HPLC is advancing rapidly in the field of pesticide residue analysis. Improvements in the performance of HPLC, ie, efficiency in separation, sensitivity, reproducibility and versatility, make this technique even more suitable for automation than GLC. The degree of automation in HPLC is increasing rapidly. The automated injection systems and microprocessors enable complete automation of the analysis. The main drawback of HPLC associated with residue analysis is the detector sensitivity. However, much progress has been reported in this field. In addition to the very sensitive UV absorbance and fluorescence detectors, improvements in other detectors are reported in the literature. The electrochemical detector was found to be very sensitive to halogenated anilines, carbamates, chlorinated and some other pesticides (Refs 79 and 80). These detectors are produced commercially by several manufacturers.

A moving-wire device that was adapted from the Pye system enabled the connection of the HPLC effluent to GLC detectors. The HPLC effluent was vaporized on the moving wire and the residues detected by any of the specific GLC detectors. The Pye moving wire has been adapted to connect the HPLC to a flame photmetric detector (Ref 81). A high-performance liquid chromatograph was connected to the Hall conductivity detector to give excellent nitrogen-selective detection and to a Tracor N-P thermionic-type detector (Ref 82). The moving-wire system was also connected to an electron capture detector and to a mass spectrometer.

On-line derivatization was introduced in order to improve HPLC selectivity and sensitivity. Krause (Refs 83,84) described an HPLC post-column fluorometric labelling technique for the determination of carbamate residues at the nanogram level. Moye discussed the possibilities for pre- and post-column derivatization of pesticide residues (Ref 85).

6. THE POTENTIAL USE OF IMMUNOCHEMICAL METHODS IN RESIDUE ANALYSIS

6.1 Introduction

Immunochemical procedures are widely used in clinical chemistry and endocrinology for the routine analysis of hormones, enzymes, viruses and drugs. In recent years, interest has been expressed in the potential use of immunochemical methods for the analysis of pesticides and related compounds in environmental samples including plants and soils (Refs 86,87). Reviews of radioimmunoassay aimed at explaining the technique to analysts have also appeared (Refs 88,89). The purpose of this report is to briefly describe immunochemical methods of analysis and discuss their application to pesticide residue analysis.

Yalow in her Nobel prize lecture traced the early development stages leading up to an immunochemical method for insulin (Refs 90,91) and emphasised the contributions that such techniques have made in endocrinology. In many cases, progress was made because of the absence of other good analytical procedures. The alternative methods such as bioassays were slow and unsatisfactory. It is now possible to assay components such as peptide hormones at the sub-picogram level as a matter of routine. However, Yalow's listing of uses in 1978 (Ref 90) heavily favoured hormones, enzymes, viruses, antigens and serum proteins, with relatively few examples of RIA methods for drugs, including cardiac glycosides, antidepressants and drugs of abuse. There were no pesticides in the list at that time. Recently, however, the potential value of immunochemical procedures in pesticide analysis has been recognised in several laboratories and an authoritative review describing this potential has been published (Ref 87).

This emphasises that there is no reason why immunochemical procedures and, in particular, RIA should not be developed for pesticide analysis and concludes that there

is every reason to expect that they would prove valuable. In general, it is likely that this limited use of RIA is due to a combination of:

(a) Conservatism - in general valid procedures by GLC or HPLC have been developed.

(b) Communication problems - the vocabulary of immunology is foreign to residue analysts.

(c) Equipment differences - some items such as γ- counters or animal breeding facilities are unavailable to residue analysts.

Although the initial steps involved in setting up an immunoassay procedure are rather lengthy, the actual analysis step can be rapid. Immunoassay kits are commerciallly available for some chemicals, to enable routine analysis to be performed and there is no reason why, if there were sufficient demand, that such kits should not be available for pesticides in the future.

Radioimmunoassay (RIA) is the most well known procedure and the stages, involved in developing a residue analysis using RIA, are as follows:

(1) Raise antibodies (Ab) to the pesticide in a suitable animal.
(2) Synthesise radiolabelled form of the pesticide.
(3) Using labelled pesticide bound to antibodies and non-labelled standards, set up a calibration curve by competitive binding.
(4) Determine specificity of the antibodies in the serum.
(5) Analyse samples.

6.2 Development of a Radioimmunoassay

1. Production of Antibodies

The first stage is to form an immunogen (pesticide derivative). When a large foreign molecule is introduced into an animal (usually the rabbit), the immune response causes its body to react forming an antibody (Ab) to absorb it. The antibody is usually specific,fitting the foreign molecule as a key fits a lock. Small molecules such as pesticides are termed "haptens" and are not immunogenic since they are normally metabolised in the animal. To induce antibodies they must be introduced into the animal covalently bound to a large protein molecule. It might be necessary to introduce a functional group into the pesticide molecule, eg, OH, COOH, or NH_2. Analogues of the pesticide may be available as a precursor or metabolite for this purpose. The hapten is then linked to a protein, typically bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) Standard procedures have been developed for hapten coupling: eg, acids can be bonded using dicyclohexylcarbodiimide; alcohols and amino groups can be first converted with succinic anhydride into hemisuccinates and these then bonded, and phenols and alcohols can be converted using phosgene via the chlorocarbonate and then linked. A summary of these techniques and full details are available (Refs 87, 92).

In designing the immunogen, it must be borne in mind that response by the animal is most likely to be good if the pesticide molecule protrudes well out from the protein so that its structure is apparent and makes good contact. It may be worth linking the pesticide through a short chain (such as the succinoyl group) to improve this. Equally the part of the pesticide to which the response is produced can be partially controlled.

The region of the molecule which is linked will normally give least response and the most exposed part will produce most. The requirements for specificity should be considered at the beginning of method development, since the choice will determine the eventual selectivity.

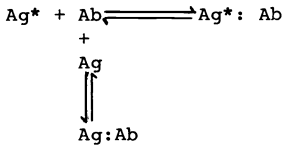
The next stage is purification of the immunogen, usually by dialysis or gel filtration to remove low molecular weight material including uncoupled hapten. The extent of loading of hapten groups to the protein then needs to be determined. If the loading is appropriately high, the immunogen is then formulated ready for immunisation of the animal to form antibodies. This application requires regular doses and the animal could take months to develop the required antibodies. The blood serum is regularly checked for antibodies which should be present in sufficient concentration after 3-6 months. Once the animal has formed antibodies, it is maintained and bled at regular intervals to obtain a stock of antiserum. One animal (such as a rabbit) will supply sufficient for a large number of analyses, since the serum is greatly diluted for analysis.

Serum samples are taken from the animal at intervals (say, weekly) for checking for antibody production and for its specificity. Specificity is usually determined by a competitive binding study and the specificity can be expressed as the concentration of a compound needed to displace 50% of an antibody-bound hapten.

Finally, when suitable serum is known to be available, a larger blood sample is taken and the serum is separated and stored for use in the analysis.

2. Developing the Immunochemical Procedure

A tracer amount of labelled antigen is used to determine the binding of unlabelled antigen to specific antibodies. The binding of labelled antigen in the presence of an unknown sample is compared with that observed with standard samples. This can be illustrated in the following competing equilibria:



where Ag represents the antigen, Ag* the labelled antigen and Ab the antibody. In the analysis system Ag* and Ab are of fixed concentrations and Ag is varied ie, as standard samples or unknowns. The separation of the antibody bound antigen from the free fraction and subsequent radioactive counting of either fraction allows the quantitation of the unknown samples by comparison with the level of radioactivity obtained from standard samples in the test system.

Once the antibody is available, development of such an RIA method is likely to be less demanding than conventional residue chemistry. In particular, development of clean-up and derivatisation procedures should be unnecessary.

Some parts of the residue method eg, the extraction stage, will probably be unchanged in immunochemical procedures. However the aim will be to obtain the pesticide in the aqueous phase rather than in organic solution as is usual in a GLC-oriented procedure. The solubility of very water insoluble pesticides can be improved by the addition of small amounts of protein (Ref 93) , and it is likely that the extract can be diluted for analysis, eg at 10ml = 1g of sample.

3. Routine Application of the Method

Immunochemical procedures can be very fast in use, especially when a substantial number of samples are run and when residues are low. They are especially definitive at proving the absence of a compound and therefore lend themselves to screening procedures (eg, monitoring water samples). Potentially it should be easier to automate radiocounting or colourimetric determinations than GLC or HPLC, especially since the materials tend to be disposable so that no equivalent of column contamination should occur. Numerical data are already often produced automatically on microcomputers and potentially could be presented as residue values.

Some regular items in the maintenance of a procedure could be:

a) Resynthesis of the radiolabel. This can be required often if ^{125}I (t1/2 = 60 day) is used, or much less often if ^{3}H (t1/2 = 12.26 year) is used.

b) Repeat preparations of antisera A highly active antserum usually obviates this requirement and sera can be stored deep frozen or freeze-dried for long periods. However, if required, new batches will need to be restandardised and their specificity to the compounds of interest must be tested. Such variations have been considered as a problem in approaching Regulatory Authorities but can be minimised by using the plasma from a number of animals or better by raising and maintaining a culture of monoclonal cells as a source of antibodies - but this is not yet a standard procedure.

c) Kits of reagents are already available from commercial sources for many analyses and could be commercialised if there was sufficient interest in their use for pesticide analysis. If this developed, then the analyst would not be involved with the rather lengthy task of preparation of antibodies.

6.3 Other Immunochemical Procedures

The Enzyme-Linked Immunosorbent Assay (ELISA)(Refs 86,87), was the subject of Al-Rubae's thesis in 1978 (Ref 95). In this alternative procedure , an enzyme is linked to the antigen or antibody and in the competitive binding assay, it is the amount of free enzyme which is determined, usually by a colourimetric method. Al-Rubae developed an ELISA method for parathion which required no clean-up of crop extracts and was highly specific for parathion. Good correlations with results obtained from GLC were obtained.

The ELISA method offers greater potential than RIA since it requires less expensive equipment and lends itself to automation (Ref 96).

Other immunochemical methods used with drugs include fluorescence techniques, free radical assays, chemiluminescence and heterogeneous enzyme immunoassay (Refs 97,98). There may also be applications in the preparation of columns loaded with anitbodies ("affinity columns") for the selective clean-up of pesticides from solutions, even though a conventional final analysis is used.

TABLE 4 Immunochemical Studies with Pesticides and Related Compounds

Compound	Reference	Comments
DDT Malathion	99, 100	Antibodies formed but not used as basis for analysis
Parathion	95	Detailed residue method worked out for parathion using ELISA. Detection limit 5.0 - 10.0 ng/ml (0.025 - 0.050 mg/kg). High specificity for parathion.
S-Bioallethrin	101, 102	Antibodies demonstrated strong stereoselectivity for S-bioallethrin.
Aldrin	93	RIA method can detect picomole amounts. Specificity based on polychlorinated bicyclic ring system, and there should be little interference from DDT and PCB's
Diflubenzuron	103	-
Benomyl and Methyl-2-benz-imidazole carbomate (MBC)	104	RIA procedure, Benomyl converted into BMC for analysis. No clean-up required from food crops. Results correlate well with HPLC method.
2-Aminobenzimidazole	105	Fluorescence polarisation immunochemical (FPI) technique.
2,4-D and 2,4,5-T	106	An RIA screening method fro detecting these compounds in ground water. Samples containing positive residues (as determined by RIA) could be analysed in detail by GC or GC.MS.
TCDD	107	-
PCB	108	A protein - binding radio-assay

TABLE 5 Advantages and Disadvantages of Immunochemical Techniques

Advantages of RIA	Comments	Disadvantages of RIA	Comments
Sensitivity	Depends on a very high specific activity of label being available.	Substantial development time	True of all immuno-techniques.
		Depends on individual animals	A problem of long-term reproductibility therefore exists.
Specificity	Can extend to stereo or optical isomers.		
		Requires new equipment	eg, radiocounters, centrifuges.
Ease of automation	Generally true.	Requires "radioactivity"	Permission and suitable procedures should not be a major barrier.
Consistency of results	Use of disposable materials avoids memory effects.	New field of activity	Training in new techniques will be required.
Low running costs	Radiocounter uses electricity but not gases as required for GC. γ-Counters are especially simple to supply and maintain.	Analysis is in an aqueous medium.	Problem of solubility with some pesticides.
(a) materials	Materials cost should not be high, expecially for γ-counting.		
(b) time	When in operation, analysis times should be lower owing to fewer interference problems, increased automation of data handling.		

Glossary of terms used in Immunochemical Analysis

Hapten:	Bound component, in this case the pesticide moiety to which the immuno response is developed.
Antigen:	pesticide - protein conjugate used to raise the antibodies.
Antibody:	Large protein (immunoglobulin) developed by immunological response with fit for the hapten.
RIA:	radioimmunoassay
ELISA:	enzyme linked immunosorbent assay (uses an enzymic colour reaction in place of a radioactive measurement).
Adjuvant:	Material added to an immunogen to enhance the immune response.
Antiserum:	Serum in which antibodies are present.
Antibody titre:	That dilution of an antiserum so that a fixed a fixed volume of diluted serum will bind a particular percentage (often chosen as 50%) of a fixed amount of labelled antigen added.
Antibody specificity:	The selectivity of an antibody for the antigen against which it was raised.
Antibody avidity:	The assessment of the amount of labelled antigen that will bind to the antibody at a given dilution. Measured as percentage bound of added labelled antigen.
Cross reactivity:	The degree of which an assay is affected by substances other than the one for which it was designed.

6.4 Application of Pesticide Analysis and Related Problems

The publications on immunochemical studies with pesticides and related compounds located in the literature up to Summer, 1981 are summarised in Table 4. This demonstrates a developing interest in this approach to pesticide analysis in several laboratories. It may be noted however, that at this stage, although a number of preparations of antigens and antibodies are described, there are limited reports of the application of full procedures.

In the fields of plant biochemistry and phytochemistry, RIA has also been used to analyse for natural products. For example, Weiler (Ref 109) developed an RIA screening method for secondary plant products (eg, digoxin) and Nickel and Staba also used RIA for assaying digoxin in tissue cultures (Ref 110). An RIA specific for the alkaloid vindoline, with a detection limit of 5 ng, has also been reported (Ref 111).

Of particular interest is the development of an RIA method for analysing sennosides (dianthrone glucosides) (Ref 112). This indicates that the method has potential for pesticide (metabolite) conjugates.

Weiler has published details of the development and application of RIAs for gibberellic acid (Ref 113), for free and conjugated abscisic acid (Refs 114,115) and for phytohormones (Ref 116). The gibberellic acid was determined in crude plant extracts. One of the abscisic acid (ABA) assays is specific for (+)-ABA with low sensitivity to (-)-ABA. It was claimed that more than 100 plant samples could be analysed for ABA in a day using the RIA.

The major advantages and disadvantages of immunochemical techniques are summarised in Table 5.

7. CONCLUSIONS

Residue analysis is entering an exciting new phase involving a range of new approaches. Chemical structures are becoming more complex and there is a need to analyse for metabolites and more polar chemicals.

Gas chromatography remains a major technique but during the past few years the use of HPLC in residue analysis has advanced rapidly.

Costs of analysis have also increased and there has been strong pressure to scale down the size of the samples used and the size of each stage in the analytical procedure. This effectively saves both time and materials as well as allowing laboratory space to be used more productively. Reduction in scale also brings the potential for some stages or all steps in the analysis to be automated. The rapid development in computers will also encourage further automation of methods. During the next few years, changes in this area will result in more cost-effective residue methods.

Many of these newer approaches will require the use of internal standards. In the analysis for single compounds and to some extent in multiresidue procedures, internal standards have already proved useful. We recommend that, provided they are chosen carefully and carried through all stages of a method, their use should be encouraged. They allow accurate recoveries to be established for each analysis and allow analysts to work more quickly with a greater number of analytical samples.

Immunochemical methods, traditionally unfamiliar to the residue chemist, also offer exciting possibilities for newer cost-effective approaches. These are only just starting to be used but will probably make a significant impact over the next few years.

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