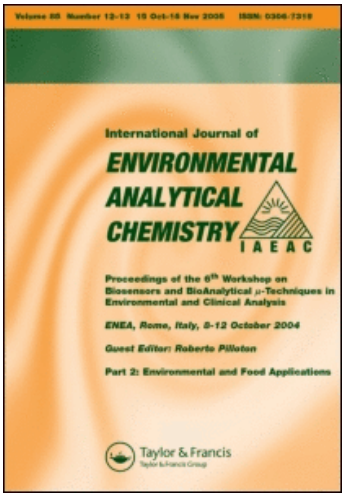

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HPLC Column Switching, a Step Toward the Automation of Sample Cleanup

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HPLC Column Switching, a Step Toward the Automation of Sample Cleanup†

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There exists a growing demand for data to meet the pesticide regulation acts and to monitor residues in food and in the environment. Speed and low running cost are very desirable properties of methods used to screen crops or commodities for pesticide residues, therefore evaluation of ways are necessary to carry out the analyses more rapidly and cost-effective. A today status of pesticide residue analysis is given. Weak points of the standard residue analytical procedures are shown. Trace enrichment and multidimensional liquid chromatography are the key-points which are prerequisites to scale down the sample size for the cleanup procedure. Reduction in scale on one hand and transfer of the individual cleanup steps, e.g. filtration, liquid–liquid partitioning, concentration, adsorption chromatography onto mini-columns or cartridges on the other hand, opens the potential to mechanize or automate the prechromatographic sample treatment.

Combination of all these different measures will reduce time and cost without the necessity to invest into expensive apparatus.

KEY WORDS: Sample preparation, miniaturization, trace enrichment, robotic sample handling.

INTRODUCTION

Pesticides have been in use for a long time. The possible presence of residues in food and wildlife is of growing public concern. To meet

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pesticide regulation today and the growing demand for data on the metabolism and degradation of the active ingredients, for monitoring residues in food and in environmental samples, an ever-increasing number of samples has to be analyzed.

This increasing requirement for residue data forces the suppliers of such data to have a close look on finding approaches to carry out extraction and cleanup steps more rapidly and cost-effectively.

Until recently, during development of extraction and cleanup procedures for pesticide residue analysis, aspects of economy where not given prime consideration. It seems that real progress in residue methodology has essentially ceased since the fundamental development in the early sixties. The analysts modified the basic concept of sample cleanup to adapt it for new compounds. In contrast to the wet chemical procedures a high degree of sophisticated chromatographic detectors has been developed since then, essentially for determining pesticides.

The goal of the residue analyst is to develop a cleanup method that isolates the residue in a sufficiently pure state for detection without serious interferences. The increasing costs force the analyst now to reflect on the financial side of analysis also. Therefore a strong pressure pushes him to scale down the size of the sample cleaned up, the volume of chemicals used and the time of analysis and to increase the overall productivity.

Reduction in scale opens new ways to mechanization or automation of residue analysis.[1]

1. RESIDUE ANALYSIS TODAY

Residue analysis proceeds in 4 steps (Figure 1) which may be optimized or automated independently.

Most space of the analytical procedures is devoted to cleanup procedures and sample preparation, so the rule "A clean sample gives the best results" seems to be still valid.

1.1 Sample preparation

Sample collection and sample preparation are time-consuming and labour-intensive, but vital operations. Pesticide residues are normally

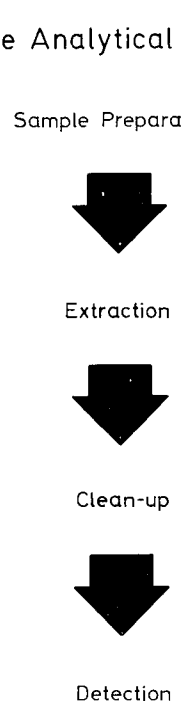

FIGURE 1 Flow diagram of a standard residue analytical procedure.

not homogeneously distributed in our sample. A small sample aliquot may therefore not be representative and may not represent the average residue concentration of the whole lot. It is necessary to collect an appropriately large sample in the field and to reduce it to a representative laboratory sample. No obvious saving can be made in this area.

Figure 2 shows an example for soil sampling. Twenty soil cores of 5 cm diameter were divided into three soil layers of 0–10 cm, 10–20 cm and 20–30 cm soil depth. Each layer of about 2–4 kg was homogenized separately.[2] Five hundred grams of these homogenized layers were then deep frozen until analysis.

1.2 Sample extraction

The final result of the analysis is directly dependent on how representative the sample was. The analytical sample is therefore taken from the well homogenized laboratory sample.

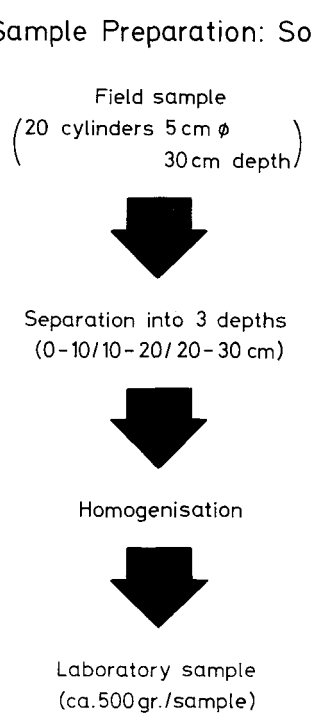

FIGURE 2 Flow diagram soil sample preparation for residue analysis.

Normally 20–100 g of sample material is used for residue analysis. This size is probably required sometimes, when the limit of determination dictates this sample size. But most often these big sample sizes are extracted simply following analytical traditions and they are not based on statistical relevance.

Looking over the residue analytical method collection of the Deutsche Forschungsgemeinschaft (DFG)[3] shows that most authors start from an analytical sample size which is much larger than needed for the final detection step. And these methods are all contributed by well established and approved governmental or industrial residue analytical laboratories. They are also tested by different independent laboratories. Therefore, we will accept them as standard routine procedure used in pesticide residue laboratories.

The methods recommend to start the analytical procedure with the extraction of 100 g of sample. Higher and lower sample sizes are

the exception. These large samples require solvent volumes of 500 ml or more for extraction. Consequently, given by the volume of the extraction solvent, the following cleanup steps require large sized glassware, space and manpower.

Table I shows the relation of sample extracted and sample size finally used for the determination by gas chromatography or liquid chromatography taken from the DFG-collection.[3] There is no reason to clean up large sample volumes just for disposal. Gas chromatographic detectors, mostly used in residue analysis, are very sensitive and specific, therefore only a small portion of the purified extract is injected for quantification. The remaining portion is rejected, a really uneconomic procedure.

TABLE I

Samples extracted and cleaned up for residue analysis and largest and smallest sample amounts used for the final determination.

	Sample sizes in GLC		
	Extraction	Detection	Waste
min.	100 g	2 mg	99.998%
max.	100 g	2 g	98%

Accurate analytical results can be obtained from very small, but properly homogenized laboratory samples.[1] The use of small sample size speeds up the following cleanup procedure and leads to an economic use of solvents.

1.3 Cleanup

Most extracts cannot be used for direct analysis and require cleanup to remove interfering substances. Multiple step methods are described, in which several purification steps are carried out before the final chromatographic detection. Figure 3 shows some generally used physico-chemical cleanup procedures.

Partitioning Aqueous miscible extraction solvents are diluted with water and the compounds of interest are partitioned into an

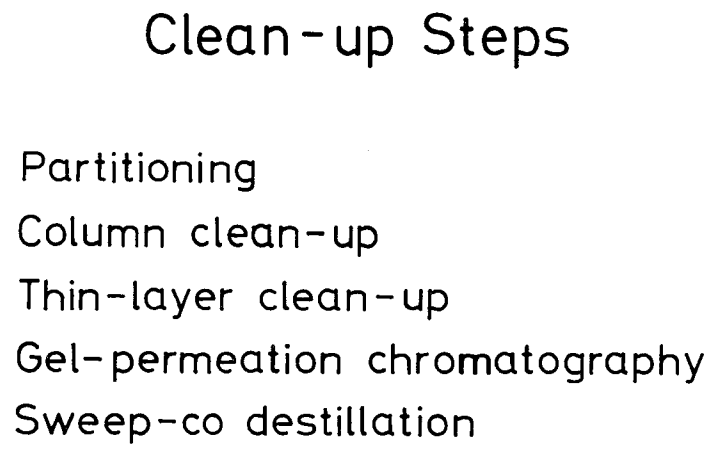

FIGURE 3 Generally used physico-chemical procedure for sample cleanup.

immiscible solvent, which is readily evaporated to dryness. Manual partitioning by shaking separatory funnels is a time consuming job. Depending on the distribution coefficient of the chemicals, several partitioning steps are necessary.

Column cleanup Adsorption column cleanup remains the outstanding feature in the purification of extracts before a chromatographic detection.

Florisil, alumina, silica etc., are the most widely used preparative adsorbents. These cleanup procedures are very effective. The activity of the adsorbent can be varied over a wide range and with an adequate solvent composition, the compounds to be analyzed are eluted with high selectivity.

Gel permeation chromatography and sweep-co distillation are very dedicated cleanup techniques, but not in general use.

1.4 Detection

The chromatographic detection techniques progressed to fully automated systems.

Fully automated detection systems include sample introduction, chromatographic separation with selective detection and data handling by computers. The wide application area of gas liquid chromato-

graphy in different analytical and research laboratories attracts equipment manufacturers and stimulates the developments. Residue laboratories turned these developments to their profit at the earlier stage.

The progress made in this area of residue analysis surpassed the development of cleanup techniques.

2. DEVELOPMENTS TOWARDS FUTURE RESIDUE ANALYTICAL PROCEDURES

Review papers [4,5] on automated pesticide laboratories are regularly published, but seldom completely new aspects or really new concepts of analytical techniques are shown. Most of the papers deal with very special problem solvings and are not of general use or cannot be transferred to the problem of other laboratories.

Promising new techniques in sample preparation were introduced in analytical chemistry recently. These techniques are not directly amenable to the most common techniques used in residue analysis today, without adaption the basic concept of residue analysis.

2.1 Miniaturization

Direct miniaturization The extraction solution will be homogeneous independent of the sample size. The following cleanup steps can therefore be miniaturized without loss of accuracy. A microchemical residue method[6] used miniaturized equipment for cleanup, to save time, chemicals and laboratory space. To handle volumes of less than 1 to 2 ml, sophisticated equipment and tools are necessary. To obtain an acceptable limit of determination the volume of the purified extract solution had to be adjusted to a definite volume of less than one millilitre. To overcome small volume handling problems, the use of internal standard methods was proposed. The internal standard method allows final solutions to be concentrated to 1 to 2 drops or another volume which is not exactly defined. Only skilled laboratory personnel can handle this small volume for injection into the gas chromatograph and no automatic sampling is possible.

The use of internal standards has not yet been commonly accepted in residue analysis, due to the free space needed in the chromato-

gram and to the difficulties to find a true internal standard with the same physical and chemical behaviour as the analyte added to the sample at the first extraction stage.

Miniaturization with the reduction of the sample size of all cleanup steps is limited to pesticides which are amenable to the most sensitive gas chromatographic detectors, the phosphorous sensitive flame ionization detector or the halogen sensitive electron capture detector.

Miniaturization as a consequence of sample enrichment An alternative solution to overcome the limitations of a straightforward miniaturization of the sample cleanup, is to change drastically the unfavourable ratios of cleaned up final volume to injected volume of sample. The potential alternative is the multi-dimensional liquid chromatography for sample enrichment, cleanup and detection.

Many examples of trace enrichment of water samples,[7–9] sample cleanup of wine,[7,15] wood compounds,[10] maize plants,[11] cereal plants and grains,[12] tomatoes,[13] fruits and vegetables[13,14] are described, but only one real combination of trace enrichment and sample cleanup by multiple column liquid chromatography was found in the literature. These authors[16] enriched the crude aqueous extract of acidic herbicides from wheat directly onto an ion exchange column of a 3-column system. Detection was done on-line with a UV-detector.

Liquid chromatography is in many ways a more powerful tool than gas chromatography and the entire potential of liquid chromatography has not yet been fully exploited in residue analysis. Many newer types of compounds appearing on the market are no more directly amenable to GC.

Halogenated organochlorines or organophosphates are easily separated and detected by gas chromatography. Carbamates, phenylureas, sulphonylureas, phenoxyacids are compounds which have to be chemically modified before gas chromatography on the other hand they can be separated by liquid chromatography without modification.

2.2 Trace enrichment

The technique of trace enrichment is a mean of sample concentration by the injection of large sample volumes onto a liquid chromato-

graphic column. Trace enrichment occurs if the elution volume of the liquid chromatographic peak is less than the sample volume injected. The loading capability of the liquid chromatographic column is usually higher than that of a gas chromatographic column. The solvent injected into the GC evaporates. The resulting sample dilution leads to an unwanted band broadening, respectively to a loss in sensitivity. Sample concentration after the injection onto the GLC column requires special injection techniques and/or special injection devices.[22] Therefore, sample volumes amenable to standard gas chromatographic systems are limited to few microlitres. This problem does not show up when injecting onto an LC-column. The sample is not evaporated while injecting into the system. We have no enlargement of the solvent volume, the chromatographic system is not disturbed if the proper solvent system is selected for injection. If a solvent system of sufficiently weak elution strength is used, the compounds of interest are concentrated on the head of the column. Sample extraction and concentration are effected on the chromatographic stationary phase.

Enrichment is needed, if the components of interest are below the detection limits. The enrichment step must be prior to the actual chromatographic separation if the components of interest are above the detection limits, but the compound of interest is present with a large amount of background interferences so that neither detection nor accurate quantification are possible.

Each sample enrichment step opens the possibility to decrease the sample amount for cleanup.

2.3 Cleanup by column switching

Figure 4 shows the general arrangement of three consecutive liquid chromatographic columns.[17] This system allows the trace enrichment, to reduce the number of sample components at the start of the analytical column. Various chromatographic "cuts"[18] can be made, prior to the analytical chromatographic step.[19,20] This procedure fractionates the sample and reduces the level of co-extracts relative to the desired components. The net result is an increase in the concentration of the components of interest relative to the background. The precolumn and the first analytical column are backflushed to clean the columns from late eluting components.

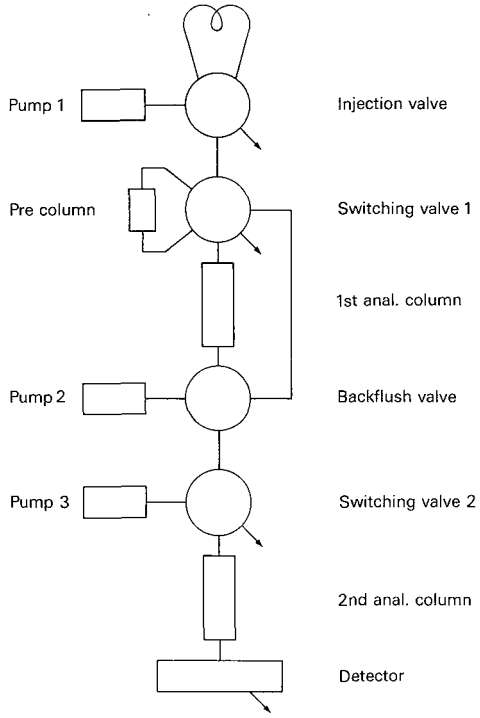

FIGURE 4 Schematic diagram of a 3-column HPLC-system.

The switching system should allow first the injection of large volumes of sample to achieve adequate sensitivity and good reproducibility of the chromatographic steps. When large samples are injected, good reproducibility may be obtained only if the analytical columns are not overloaded. Separation and sensitivity are improved if sample dilution on the columns are minimized throughout the analysis. This involves reconcentration of the components zone almost at the top of the analytical column. Reconcentration is achieved by using columns with successively stronger retention and with mobile phases with increasing elution strength.

Combination of a trace enrichment step followed by multidimensional chromatographic separation steps fills one of the gaps towards the demanded better sample exploitation.

2.4 Online multidimensional LG–GC

Offline liquid chromatography is a frequent cleanup technique before the gas chromatographic determination. Detectors sensitive to nitrogen, phosphorous or halogens are widely used in trace analysis by gas chromatography. Liquid chromatography lacks such a wide variety of different selective detectors. The combination of the unique potential of liquid chromatography, e.g. trace enrichment and multidimensional separation, with the selective detection of gas chromatography, reveals a potent dimension in automated trace analysis. The disadvantage of gas chromatography, the low sample volume which can be injected, will override the selectivity of the detection.

A nearly symmetrically formed peak (Figure 5) meets the sample concentration profile of the chromatographic effluent and is approximated by a triangle. The top fraction of few microlitres is injected into the gas chromatograph. The amount of sample transferred is calculated by twice the amount injected into the liquid chromatograph, multiplied by the volume injected and divided by the total volume of the peak effluent.

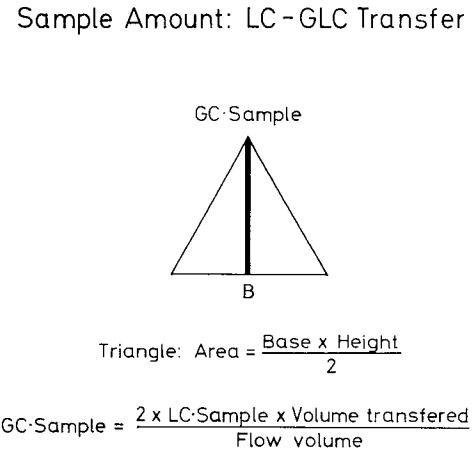

FIGURE 5 Calculation of the approximate sample transfer LC to GC.

Suitable interfaces[18,20] are available in the form of liquid samplers for gas chromatography with flow through side arm syringes. The volumes injected into the gas chromatograph are only few microlitres.

Grob Jr and collaborators[21,22] showed an interface technique where several hundred microlitres of the LC effluent are introduced into a capillary column. The sample enrichment occurs in the flooded inlet by slow evaporation of the solvent.

2.5 Prechromatographic cleanup

All or part of the usually necessary cleanup can be transferred to the multicolumn liquid chromatographic system. A prechromatographic sample treatment, more or less extensive and time consuming may often be necessary nevertheless to bring the sample into an appropriate solution for injection. Procedures such as liquid–liquid extraction, column chromatography and evaporation are commonly applied.

Liquid–liquid partitioning, usually made by manual shaking can be transferred onto columns filled with Kieselgur (e.g. Extrelut® and Extube®, Clin Elut® are commercially available prepacked columns from E. Merck AG, Darmstadt, F.R.G., or Analytichem International, Harbor City, CA, U.S.A.).

Disposable columns or cartridges of relatively low cost are available filled with a wide variety of chemically modified silica gels, ion exchange resins and adsorbents. The cost of these columns may easily be recovered by the time and solvent savings. The high priced chemically modified silica gel prevents those adsorbents from being used in standard sized non-regenerable preparative columns.

These new mini-columns remove very efficiently crude coextracts or may be used to concentrate the compounds of interest without evaporation of solvent.

Designed primarily for cleanup of low volumes of biological fluids, they meet the requirements of the prechromatographic sample treatment in residue analysis also, provided the liquid chromatographic enrichment technique is applied to reduce the sample to an appropriate size.

3. AUTOMATION OR MECHANIZATION

Ideally, analysts want access to an analytical instrument that will accept unmeasured, untreated sample at one end and provide a full

report, in correct concentration units, at the other end, requiring no operator involvement beyond keeping the reagent bottle filled.

Recent developments in gas and liquid chromatography provided the basis for what we call automation today. These improvements include new column technologies in gas and liquid chromatography, automated injection systems and online computer systems to collect data, to control the operation of the chromatographic system, and finally to process the data.

Automation of a laboratory means far more than computerizing the chromatographic detection. Automation also includes the problems associated with the sample movement and manipulation of samples. Less progress in this prechromatographic sample treatment was registered.

In the analytical laboratories samples are crushed, ground, extracted, aliquoted, diluted, concentrated, filtered etc.

Automated dedicated sample processors can be implemented as a part of fully automated systems or as stand alone semi-automated instruments to simplify one aspect of an analytical procedure.

3.1 Concepts in sample treatment

Two main concepts have been applied with varying success. The first concept which is very popular in clinical and chemical laboratories is the flow analysis concept. The segmented or non-segmented liquid streams from one module to another, each of which automatically carries out a different wet chemical analytical function.[23]

The second, discrete or batch concept uses individual sample containers. The reagents are added consecutively in the required proportions, mixing, filtration, extraction and the like are accomplished in the sample container. These discrete analysers or modules have a high throughput. They are not fixed into one analytical system. Interfacing the different modules together to a fully automated system is the most difficult and costly step.

A careful study of the individual prechromatographic cleanup steps involved in the majority of residue methods, reveals some basic operations which are executed in all laboratories within most residue procedures. Mechanization which complies with this standard methodology should be easy to develop, not be very expensive and versatile with respect to different samples.

After solid samples are weighed, it is customary for them to be treated in such a manner that they can be handled as liquid throughout the remainder of the sample preparation scheme.

Transfer of physico-chemical different cleanup steps on to low resolution disposable columns reduces different cleanup steps to the same mechanical operation.

Physical cleanup steps which may be run on columns:

—removal of insoluble particles, e.g. filtration,
—removal of solvents, e.g. evaporation, concentration,
—separation by chromatography or by liquid–liquid partition.

In the separation procedure, sometimes the analyte is retained for elution after interfering materials have been washed out. This procedure will also exchange the solvent or circumvent the solvent evaporation step. Otherwise the compound of interest passes through the column, while solid particles and/or excipients are retained.

Separation by partition between to immiscible liquids are much more efficient when transferred onto extraction columns.

3.2 Samples handling system

Robots, new laboratory equipments, are multifunctional manipulators. They are capable of moving a variety of tools and parts through a variable, preprogrammed task. The robotic workstation includes three components (Figure 6), the mechanical handling arm, the robot; the control unit or computer and the chemical instrument. The chemical instrument may be, e.g. a simple mixer or a self-contained workstation, fed by the robot with samples.

Robots are applied to a wide range of automation problems, from simple manipulation of test tubes to complex sample preparation and derivatization schemes.[24,25]

In most cases the benefits of the robots do not result from the fact that the robot performs the work faster than its human prototype. It is usually slower. The productivity gains are made if the robots will run longer and they will make fewer mistakes than human workers.

Robots are often used with too many degrees of freedom, just to copy the operation formerly carried out manually.

Robotic power is efficiently used when the repetitive jobs are delegated to independent workstations and the robot transports and

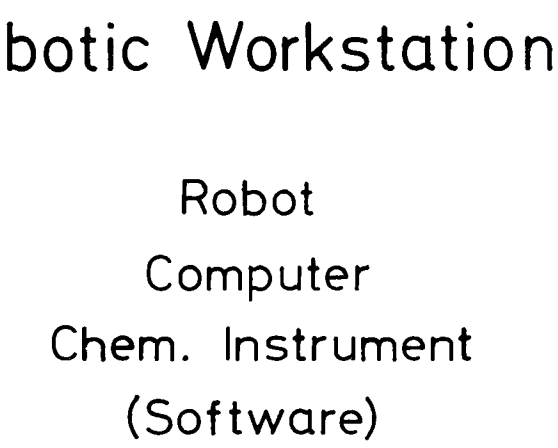

FIGURE 6 Modules to set up a robotic workstation.

handles the sample between the different workstations. Single arm manipulators with Cartesian movement capability (Figure 7) together with diluter/dispenser units may be composed to powerful fast operating workstations for mini-column handling (Figure 8). The syringe system can be used to dispense reagents and aliquot samples for diluting or partitioning. Syringe A pipettes a sample aliquot to

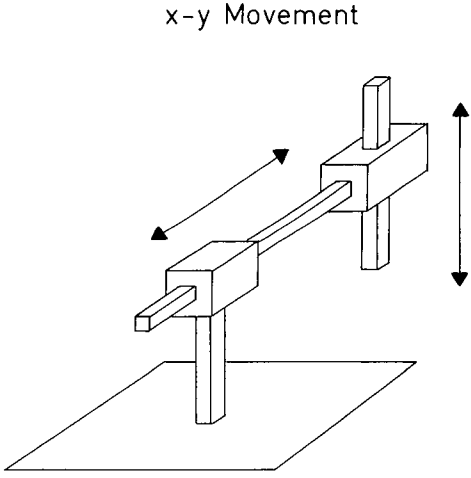

FIGURE 7 Schematic function of handling unit with *x-y* movement.

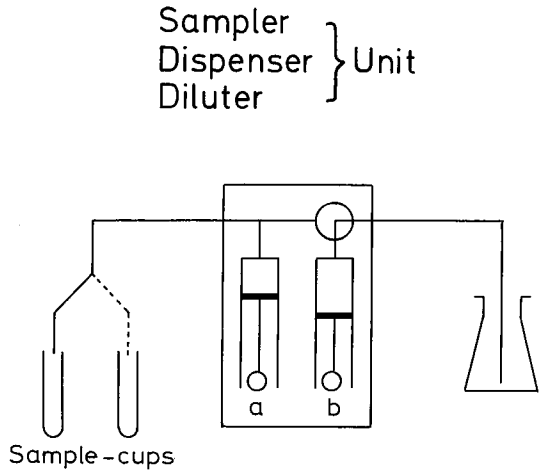

FIGURE 8 Schematic function of dispenser used within column cleanup workstation.

dispense it in the receiving flak, while syringe B will rinse the pipette. If the receiving flask is a mini-column the workstation can be used for sample filtration, concentration or partitioning.

With this modular configuration, the analyst selects only the modules required to perform a particular task. An intermodule sample transport by robot will upgrade the system to full automation. It may not always be economically feasible to pursue total automation. The flexibility in the offline use of discrete self-contained modules will outweigh often the disadvantages.

CONCLUSION

Sample handling in pesticide residue analysis contains a great potential of rationalization, provided we will be able to reduce the sample size for cleanup on a statistically evaluated basis and we enrich online the compounds of interest before the chromatographic detection.

Combination of these different measures lead to timesaving and cost effective residue procedures, without the necessity to invest into expensive apparatus.

Residue analysts have to turn away from old rutted ways to miniaturized and economic procedures.

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