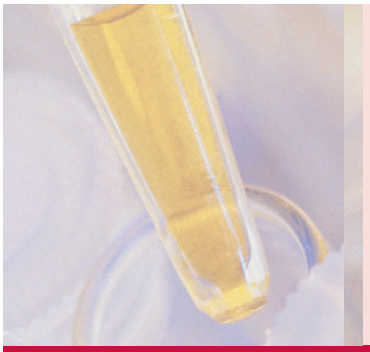

Laboratory Products Focus

IMPROVING ANALYSIS OF PESTICIDES

A NEW METHOD DEVELOPMENT PROTOCOL TO INCREASE RECOVERY OF VOLATILE COMPOUNDS

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Determination of pesticide contamination of fruit and vegetables usually follows several steps, including, extraction of analytes from the sample, concentrating the extract, post extraction clean-up, solvent exchange, and finally, determination of the analytes present.

During sample preparation, evaporation becomes a critical step when the pesticides are semi-volatile compounds, because some of the compound may be lost during the concentration and evaporation stages. Analyte loss is detrimental to accurate analysis, and the official directives(1) must be satisfied with regard to minimum analyte recovery. Typically a minimum recovery of 70% must be achieved.

In order to evaluate new instruments and improve productivity, which is also the remit of ARPAT, this study was done to determine how a new generation centrifugal evaporator may simplify the sample preparation process and improve analyte recovery.

The study falls in two halves, first, an evaluation of the a new evaporator to determine the optimal concentration process; then, the study was addressed towards real matrices spiked with pesticides to validate the new methods. The new processes and methods were then compared to the original method validated in ARPAT laboratories.

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EXPERIMENTAL

Analytical method development involves the careful evaluation of instruments with regard to issues such as ease of use, efficiency, and most importantly analyte recovery and integrity. In this study the performance of a new centrifugal evaporator manufactured by Genevac, U.K., is evaluated.

The stages of the research methodology are as follows:

- Determination of which analytes are suitable for the study, in terms of volatility and recovery, to be used as probes.
- Evaporation tests on the solvents used for the extraction of pesticides from fruit and vegetables.
- Development of an optimal evaporation method using the EZ-2 ENVI evaporator, which avoids unacceptable loss of pesticide and delivers a concentrated sample, rather than a dry sample.
- Validation of sample preparation method using a test matrix spiked with pesticides, relative to the existing method previously validated and adopted by ARPAT laboratories.

DESCRIPTION OF THE EZ-2 ENVI

The EZ-2 ENVI (shown in *Figure 1*) is a state of the art centrifugal evaporation system, which allows highly controlled evaporation and concentration of samples. Vials, tubes, microtitre plates and flasks can be accommodated in the system. During evaporation, samples are centrifuged in order to avoid "bumping" effects which may result from boiling under vacuum. Sample temperature, vacuum level, and heating controls are all precisely monitored and controlled throughout the evaporation or concentration process. The software also controls start up and can determine the end of evaporation automatically.

Figure 1. EZ-2 ENVI, The State of the art Centrifugal Evaporation System

SAMPLE CONCENTRATION METHOD

The evaporation method may be set to run for a fixed time in order to stop the system when we estimate that we have reached a certain residual volume in each tube. This method was found to be unreliable, therefore a "solvent keeping" approach was trialed where a 1ml aliquot of toluene was added to each sample. Toluene (boiling point 110°C) is less volatile than ethyl acetate (boiling point 77°C) in which the samples were dissolved. The object of the study was to verify if pesticides could be successfully concentrated into the residual toluene by a fractional distillation method, thereby removing only the ethyl acetate. Toluene was selected not only for the marked difference in the boiling point to ethyl acetate, but also for it's excellent solvatation properties of most pesticide chemical structures.

The task was to build an evaporation program which avoids complete drying of the sample, which would be detrimental, but which allows automatic reduction to a fixed solvent volume with no user intervention. All the evaporation methods were tested with samples contained in 12 flat bottom ASE collection vials, 27.5 mm diameter. Six were placed into each of two aluminium sample holders from the machine. Two pre-programmed methods were used in succession, first the 'Enviro' program which reduces the pressure gradually with two vacuum gradients to prevent bumping, then the very low boiling point solvent program, which is designed for safe removal of very low boiling point solvents. The temperature of the sample holders was programmed to be 30°C. The combined method profile is as follows:

1. Vacuum gradient from 600mbar to 200mbar in 10 minutes, at ambient
2. Vacuum gradient from 200mbar to 150mbar in 15 minutes, at ambient
3. Hold pressure at 75mbar for 65 minutes, holder temperature 30°C.

The method was developed based on the hypothesis that toluene will not evaporate under these conditions, but ethyl acetate will. At 75mbar ethyl acetate's boiling point it is 16°C while the boiling point of toluene is 40°C. Keeping the holder temperature at 30°C will mean that there is sufficient energy to boil the ethyl acetate, but not the toluene. In this way, the large volume of ethyl acetate is safely removed leaving the analyte in the toluene.

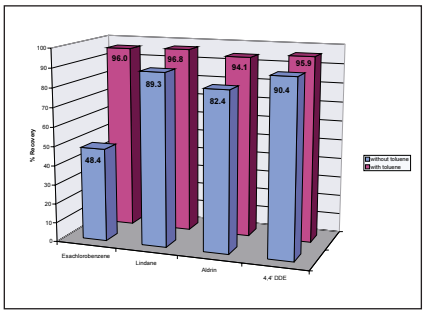

Figure 2. Pesticide recovery following concentration from ethyl acetate, with and without addition of toluene

Figure 2 shows recovery data obtained using this method. A Pesticide mix containing very volatile compounds (Hexachlorobenzene, Lindane, Heptachlor, Aldrin 4,4' DDE, Endrin) was spiked into 50ml ethyl acetate samples in ASE collection vials, with and without the toluene addition. The tubes were then dried according to the method outlined above and the recoveries determined using GC-ECD. The results confirm the hypothesis: samples in ethyl acetate were completely dried and recovery was reduced, where as under the same conditions, samples containing toluene were reduced to a small volume - about 100µl – and had very high recovery rates.

To further investigate the suitability of this approach, the stability of pesticide in the residual toluene at 30°C and 75mbar was evaluated. 2ml Vials containing 1ml of toluene were left for different period of times at 30°C at pressure of 75mbar. To facilitate analysis, a molecule with a high molecular weight was also spiked into the samples, endosulfanlattone, which will not evaporate. Pesticide concentrations were determined by GC-ECD and measured relative to the concentration of endosulfanlattone.

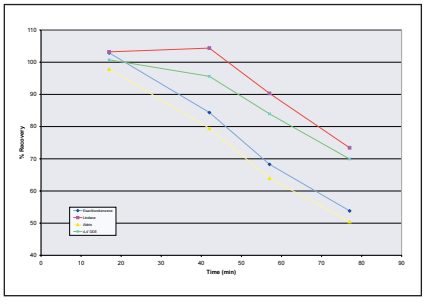

Figure 3. Stability of pesticides in toluene under vacuum

The results are shown in *Figure 3*. We can see that the evaporation of toluene at 30°C, 75mbar occurs very slowly. After 30 minutes of centrifugation under these condition there is, for some analytes, a unacceptable reduction in recovery. The relationship between the weight reduction of toluene and the reduction in the recovery of the pesticides seems to be significant.

In order to investigate this further, another stability test of pesticides in toluene was performed. This time, instead of referring to the internal standard, endosulfanlattone, vials containing pesticides in 1ml toluene were weighed before and after the evaporation step. Vials were exposed to the same conditions as the previous test, 30 minutes at 30°C and at 75mbar. The aim of the test was to validate the concentration against the weight and correlate recovery loss to evaporation of toluene.

The results are shown in *Figure 4*. We can see that after 30 minutes there was no significant reduction of toluene volume (less then 1%), and an acceptable recovery of pesticides.

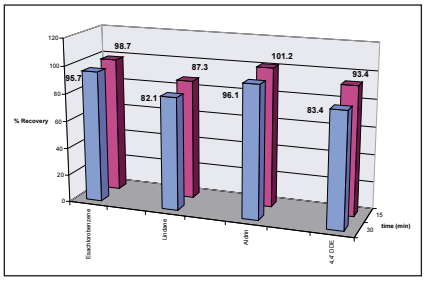

Figure 4. Recovery of pesticides in toluene following exposure to vacuum

Based on this information, we have further developed the EZ-2 ENVI to automatically end the evaporation process within 30 minutes. From this data we can see that 30 minutes exposure to the conditions of 30°C at 75mbar, is the maximum for the 4 pesticides tested. These 30 minutes are useful to ensure the complete evaporation of ethyl acetate from all samples so as to leave them only in the toluene. Another benefit of the addition of toluene is to reduce variations in evaporation speed caused by differing numbers of samples in different runs, where one sample may dry before another.

EFFECTS OF WATER IN THE SAMPLE

When working with real samples, water from the organic matter may carry into the ethyl acetate extracts; therefore it is possible to obtain a binary mixture of toluene and water. These solvents form an azeotrope whose boiling point is close to that of ethyl acetate, therefore the solvent keeping approach would not work. For the following experiments anhydrous sodium sulphate was added directly to the samples in the ASE vials, before the evaporation step to dry the samples, and avoid the potential formation of an azeotrope. Water is also detrimental to chromatographic analysis, and should be removed. This approach is a faster method for drying the extracts than filtering them through a sodium sulphate septa. Once sodium sulphate adsorbs water it will be compressed by the high g-force and stick to the bottom of the vials. The liquid sample of toluene is then easily removed leaving the sodium sulphate pellet in the base of the tube.

A NEW MORE EFFICIENT SAMPLE PREPARATION METHOD

Taken together, this information has helped us to develop a new sample preparation and evaporation method suitable for EZ-2 ENVI, effective for fast solvent removal without significant loss of pesticides.

The new method is detailed below. The method fractionally distils the ethyl acetate/toluene mixtures using a vacuum ramp, and maintaining sample temperatures below 30°C.

At the end of the method the pressure is increased to 150mbar to offset any increase in ambient temperature which may contribute to loss of sample.

1. Extract samples in ethyl acetate 50ml
2. Add 1ml toluene to each sample
3. Add anhydrous sodium sulphate to each sample
4. Concentrate using the following method: Vacuum gradient from 125 mbar to 75mbar in 25 minutes to evaporate gently. Evaporation continues at 75mbar at 30°C for up to 60 minutes.

Pressure rises to 150mbar to prevent further sample loss for up to 30 minutes.

VALIDATION OF STUDY USING LETTUCE MATRIX

The new approach was tested using samples of lettuce spiked with a the same mix of volatiles pesticides (Hexachlorobenzene, Lindane, Heptachlor Aldrin 4,4' DDE, Endrin).

The full process is detailed in figure 5. The original sample preparation and testing method is shown to the left, and the steps required with the new method are shown to the right. Common steps are in the middle.

Two series of lettuce samples were analyzed, each sample being 10g of homogenised lettuce spiked with a 200µl mix of pesticides each at 5ppm concentration. The final pesticide concentration for injections was 0.2 mg/l, equal to 0.02mg/kg in lettuce matrix.

The recovery achieved for all the pesticides tested exceed the statutory minimum of 70%, including for very volatiles pesticides, like Hexachlorobenzene. The results are shown in *Figure 6*.

The two series of samples were tested applying the same method, the second series of samples (show in red in *Figure 6*) were performed by adding 1ml toluene at both evaporation steps, while the first one (shown in blue) was performed by adding toluene only in the first evaporation step. It is evident that the series where toluene was added to both evaporation steps delivers a better recovery – which supports the data presented in *Figure 2*.

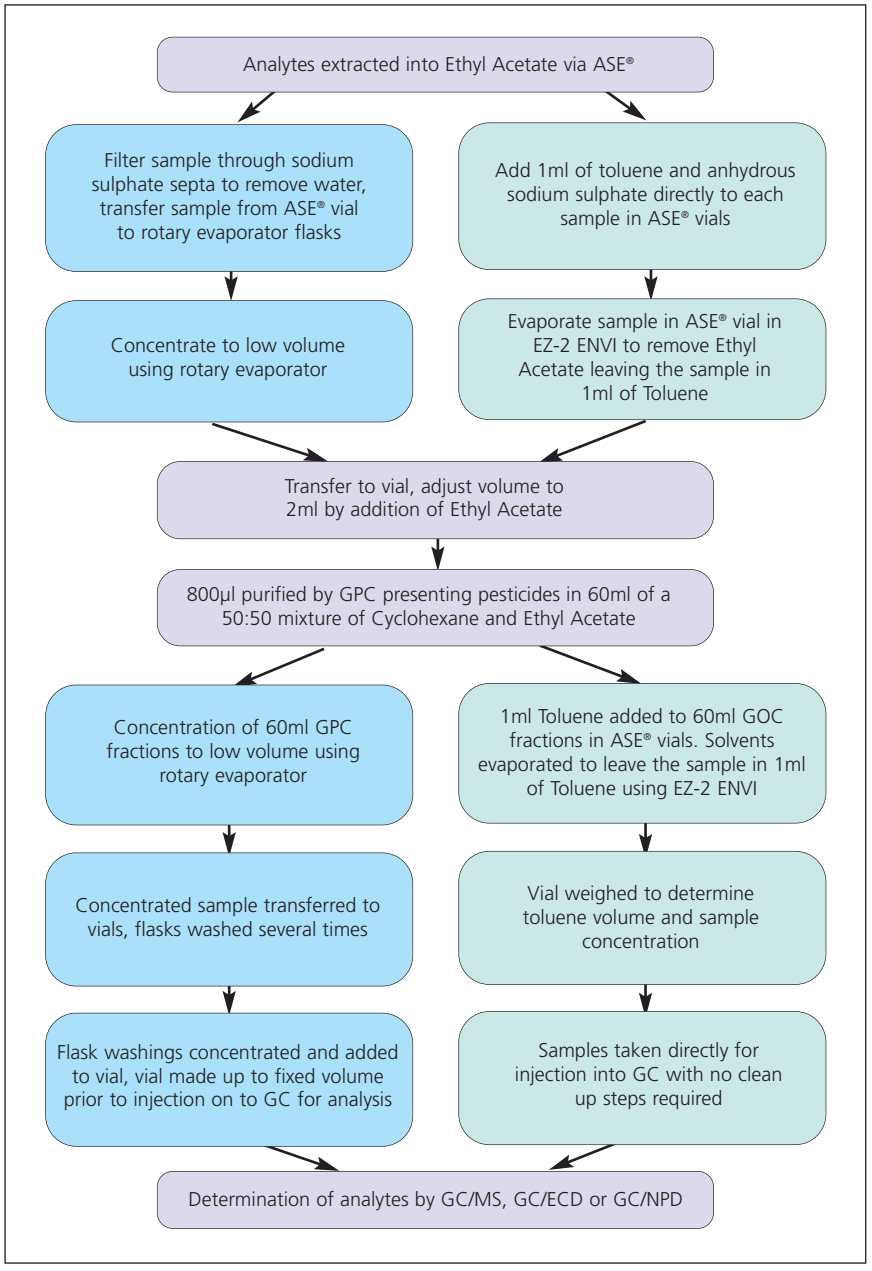

Figure 5. Sample preparation and testing methods for pesticide residue and testing. Steps of the original method are shown to the left, steps for the new method to the right, common steps are in the middle.

Standard deviation on the recovery obtained by three repetitions was very low for both series of tests. Where toluene was added to both evaporation steps, the standard deviation was under 3%.

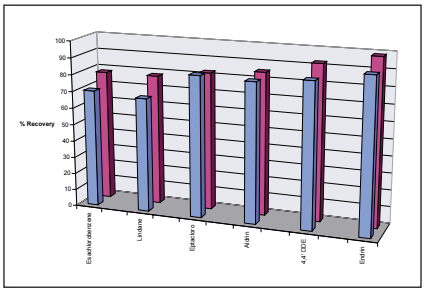

Figure 6. Recovery data on lettuce samples spiked with pesticides, extracted by ASE system and analyzed following the modified method

CONCLUSIONS

The new method developed at ARPAT using the new evaporator delivers satisfactory recovery and reproducibility for the analysis of volatile pesticide compounds extracted from organic matrices. From a practical point of view it is evident that reducing the number of sample transfers between different containers, and the elimination of the filtration step by addition of anhydrous sodium sulphate directly to the samples, is a significant advantage.

The EZ2-ENVI concentrates a number of samples at the same time, and provides protection from cross contamination and bumping with accurate temperature and vacuum control.

This provides hands free, fully automated concentration to the analyst, compared to use of a rotary evaporator, and delivers a great productivity gain. The EZ-2 ENVI also has a serial port and evaporation data may be downloaded for quality control purposes.

1. Quality control procedures for pesticide residues analysis; Document N° SANCO/10232/2006 24/March/2006

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