ANALYTICAL METHODOLOGY OF PESTICIDE RESIDUES IN THE FINNISH CUSTOMS LABORATORY

KALEVI SIIVINEN

Laboratorio de Aduanas
Helsinki. Finlandia

One of the main duties of the Finnish Customs Laboratory is to provide consumer protection by its analyses of imported foods. The determination of pesticide residues has an important role especially in the control of fresh fruits and vegetables.

The large number of shipments, which should be inspected for pesticide residues, sets certain criteria for the analytical methods. The methods must be fast, reliable and relative simple. Furthermore, as many pesticides as possible should be covered by the methods.

1. Multiresidue methods

1.1 Sample extraction

The wide variety of different pesticides, which may be present in the inspected shipments, necessitates the use of multiresidue methods. For most non-fat foods and especially for fresh fruits and vegetables we use the Luke procedure with some modifications (Luke et al., 1975, 1981). In this method the sample homogenate is extracted with acetone. The effectiveness of the extraction is based on the presence of water in the sample itself. Thus, before extraction water must be added into dry samples like rice and cereals.

The acetone extract is then partitioned by shaking with petroleum ether and dichloromethane to separate the water.

The separated water is saturated with NaCl and extracted twice with dichloromethane. The combined organic solvents are then evaporated to small volume by rotary evaporator. Finally a small drop of keeper solution is added to avoid the loss of the most volatile compounds and the residual solvents are removed by nitrogen blow. The residue is then dissolved into acetone for gas chromatographic analysis.

With this method most of the organohalogen and organophosphorus pesticides, as well as carbamates and triazine herbicides will be extracted with good recovery. In principle the extract will contain

residues of most of the non-ionic pesticides.

However, the recovery of the most polar pesticides may be incomplete. For example the recovery of methamidophos is poor and variable. The reason for this is probably the fact that in the partition methamidophos tends to remain in the water layer. To avoid this problem, the replacement of petroleum ether by acetone in the partition has been used. This procedure seems to give an almost complete recovery for methamidophos. Also the amount of coextractives increases, thus making the analysis of this extract impossible with nonspecific detectors like electron capture (EC).

However, successful analysis can be done with nitrogen and phosphorus sensitive detection (NP).

In our laboratory we have also observed that good recovery for several pesticides will be achieved with only one single partition step. In this method the partition is carried out simply by shaking the acetone extract with petroleum ether, dichloromethane and sodium chloride for two minutes in the separatory funnel. By omitting the two other partitioning steps considerably time is saved. Thus this method is used sometimes to enhance sample throughput.

Table 1 shows the comparison of recoveries with the single partition method and with the normal Luke procedure.

1.2 . Sample clean up

In their original method Luke et al. (1975) used florisil clean up before analysis with EC to avoid disturbing sample peaks. However, later they totally omitted this clean up.

This was possible because they replaced the EC detectors by more specific Hall electrolytic conductivity detectors (HECD). In our laboratory we use EC detectors for halogen compounds and inspite of this several samples can be analyzed without additional clean up. However, certain commodities e.g. onions, lemon, grape fruit and carrot, show very severe background peaks. For these samples we use sorbent extraction clean up. The sample extract is run through a

prepacked octadecyl cartridge in a methanol water mixture and the pesticides which are trapped by the column are eluted out with hexane. This procedure works well for non-polar organohalogen compounds.

1.3. Gas chromatographic analysis

The analysis of Luke extracts is usually made by gas chromatography (GC), although some experiments have been made to increase the number of detected compounds by use of high pressure liquid chromatography (HPLC), too. The GC technique is superior for volatile compounds, which are thermally stable. However, the chemical structure of pesticides is so diverse that several different liquid phases have to be used. Inspite of this some compounds doesn't elute ideally at least when nanogram amounts must be detected.

The superiority of GC is based on the specificity of the detection. We use nitrogen and phosphorus sensitive thermal ionization (NP) detectors and EC detectors. The specificity of the NP detectors is excellent, but for EC detection an extra clean up of the sample extracts is sometimes needed.In the HPLC technique derivatization is usually necessary to get comparable sensitivity and specificity.

Both packed and capillary column are used in the GC analysis. The old packed column technique has still some advantages, which support the use of it. The selection of different liquid phases is large and the injection directly into the packing is simple and gives a good quantitative reproducibility. On the other hand the better resolution of capillary columns makes them superior especially in the analysis of complex matrixes where disturbing sample peaks occur.

In the capillary technique we use retention indexes for the qualitative identification. The accuracy of the calculated indexes is achieved by adding the retention index standard into the sample. The identification is accepted if the calculated index value fits to the known index values of the compound on two different stationary phases.

Quantitation is made by use of internal standard. This eliminates most of the inlet related problems.

Confirmation of the GC identifications is sometimes made by mass spectrometry. Especially, when residues exceeding the maximum allowed level are found. Both selected ion monitoring (SIM) and full spectrum scanning techniques are used.

1.4. Automation of the gas chromatographic analysis

The high resolving power of capillary columns makes possible the automation of the GC analysis. We are using dual channel chromatographs equipped with two columns of different polarity and a commercial chromatography software (HNU-Nordion) for the automatic analysis (Figure 1).

However, the application of the software into pesticide analysis has been developed in our laboratory (Kiviranta, 1987).

The sample and the internal standards for index and quantity calculations are injected together at the same time into the memory of the attached computer. The computer calculates retention index for every peak in the chromatograms and compares them with the index library file. Only those compounds, which are positively identified on both columns will be reported. The quantitation is also made automatically by use of internal standard and relative response factors.

A three point calibration is stored into the computer memory for most common pesticides. The total number of different pesticides in the library file exceeds 150.

2 . Dithiocarbamates

Dithiocarbamates are a rather old group of pesticides, which are still widely used, possibly because they may be less prone to the development of resistance than many newer fungicides. In some countries their use has been restricted, because of the concern about the

carcinogenic metabolite (ETU) of ethylenebisdithiocarbamates.

The residue analysis of dithiocarbamates is not possible by the gas chromatographic multiresidue method. Instead a spectrophotometric method is used (Winell, 1975).

The dithiocarbamates are hydrolyzed by refluxing with hydrochloric acid and the liberated carbon disulphide is trapped and allowed to react with the color reagent.

The intensity of the formed yellow color is measured.

This method was tested recently in Finland in an intercalibration. This study showed that the recovery varied between 63 and 85%, thus necessitating the use of correcting factor. Our experience, however, shows that the method work in routine analysis,.if care is taken to avoid leaks in the refluxing apparatus. For that reason we prefer the use of vacuum instead of nitrogen blow to carry over the liberated carbon disulphide. The method is also very rapid and suitable for most commodities.

3. Benzimidazole fungicides

Benzimidazole fungicides - benomyl, carbendazime, thiophanate methyl and thiabendazole - are used for pre- and post-harvest treatments of several crops.

The analysis of these compounds is carried out by alkaline ethyl acetate extraction of the sample homogenate, and clean up by liquid - liquid partitioning (Mestres et al., 1974).

Benomyl and thiophanate- methyl are converted in this method to carbendazim. Thus, the spectrophotometric determination of carbendazime measures the sum of these three compounds.

The reliability of this method has been increased in our laboratory by recording the second derivative of the UV- absorbance spectrum. This allows accurate determination even in the presence of disturbing background absorption.

Thiabendazole can be measured by also UV-spectrophotometry,

but a more specific and sensitive detection is achieved by fluorimetric detection.

We have also started the HPLC screening for these compounds, but so far only the analysis of thiabendazole works routinely.

4. Fumigants

4.1. Inorganic bromide

Methyl bromide is widely used for soil de-infestation and for fumigation of stored crops. Methyl bromide decomposes rapidly to inorganic bromide, and the residue control of this fumigant is usually done by measurement of the degradation product.

We have analyzed inorganic bromide for several years with the gas chromatographic method of Stijve (1981).

In this method the bromide ion is converted to 2-bromoethanol by reaction with ethylene oxide in an acid sample slurry, whereupon 2-bromoethanol is then extracted with ethyl acetate. Direct gas chromatographic analysis is then made by drying a small aliquot of the ethyl acetate layer and by injecting it into a Carbowax column. EC detection is used to enhance the selectivity.

We have thoroughly tested the method in routine use during eight years. Several thousands samples - cereals, nuts, almonds, dried and fresh fruits and vegetables - have been analyzed. Comparison to other methods (HPLC and X-ray fluorescence) has been made. The method has proved to be accurate and reproducible.

4.2. Ethylene chlorohydrin

Ethylene oxide (EO) is used for sterilization of certain foods like dehydrated vegetables and spices. However, EO is very volatile and reactive compound and the residue disappears rapidly after treatment. EO reacts with the inorganic chlorides present in the product,

yielding ethylenechlorohydrin (ECH), which is fairly stable.

To analyze the ECH residues we use a modification of the solvent extraction method of Stijve et al. (1976).

Instead of acetonitrile - water mixture we use acetonitrile - methanol (5:1) to extract the residue. Furthermore, the analysis is carried out by polar capillary column (OV-351) instead of packed column to get better separation of the analyte from sample peaks. 1-Hexanol is used as internal standard to eliminate injection errors.

4.3. Hydrogen phosphide

Aluminium and zinc phosphides are used as fumigants during storage and transport of different crops. The hydrogen phosphide (PH_3) gas, which is liberated from the salts has a strong insecticidal effect. A gas chromatographic head space technique is used to detect the residues. The residue is liberated from the sample by acid and extracted into toluene in a tightly sealed head space flask. The rapid analysis is carried out by direct injection of the head space gas into a capillary chromatograph equipped with NP detector.

5. Miscellaneous methods

Occasionally some other methods are also used in the control of imported foods, depending on the use of pesticides in the country of origin.

Quite regularly, however, residues of diphenyl and 2-phenylphenol are analyzed. These fungicides are used for post-harvest treatment of citrus fruits and apples.

Steam distillation of the compounds and simultaneous GC analysis is carried out (Pyysalo et al., 1978).

Residues of daminozide (a plant growth regulator) have been analyzed for several years in apples with the spectrophotometric method. However after the potential carcinogenity of its UDMH - metabolite was reported, we started to use the more sensitive mass spectrometric method (Conditt et al., 1988) and more effort has been directed to the analysis of processed apple products like juices.

Litterature

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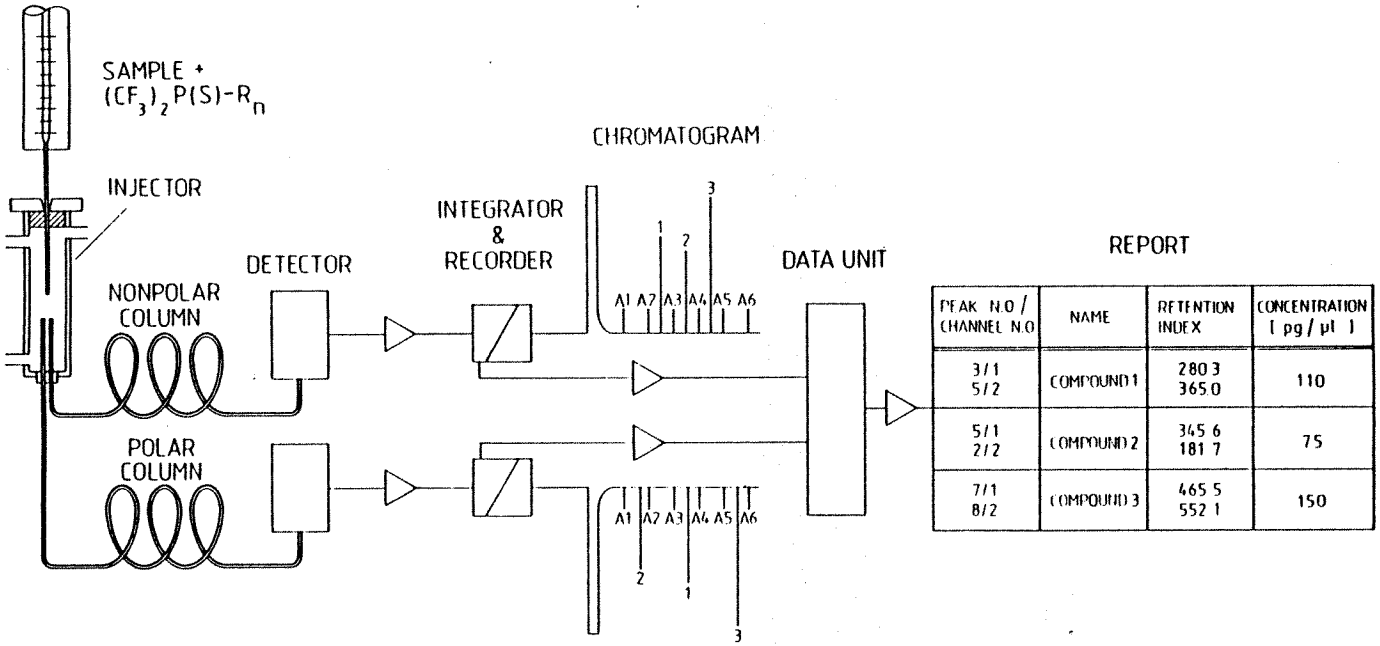
Table 1. The recovery of some pesticides by the single partition modification of the Luke method compared to the original procedure.

Pesticide	Single partition Recovery %	Luke procedure * Recovery %
Acphate	78 (pepper)	111 (tomato)
Captan	82 (pepper)	105 (tomato)
Chlorpyrifos	94 (pepper)	99 (green bean)
-Endosulfan	90 (pepper)	93(cucumber) **
Ethion	94 (pepper)	97 (lettuce)
Parathion	87 (pepper)	107 (celery)

* Luke et al. (1975, 1981)
** with florisil clean up



PEAK N.O / CHANNEL N.O	NAME	RETENTION INDEX	CONCENTRATION [pg / µl]
3/1 5/2	COMPOUND 1	280 3 365 0	110
5/1 2/2	COMPOUND 2	345 6 181 7	75
7/1 8/2	COMPOUND 3	465 5 552 1	150

Figure 1. Operational principle of the automated GC system.