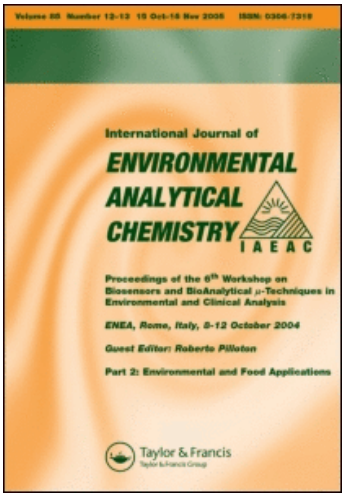

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Ion Trap Detection for Development of a Multiresidue/Multi Matrix Method for Pesticide Residues in Agricultural Products

L. G. M. Th. Tuinstra[a]; P. Van De Spreng[a]; P. Gaikhorst[a]
[a] State Institute for Quality Control of Agricultural Products (RIKILT-DLO), AE Wageningen, The Netherlands

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ION TRAP DETECTION FOR DEVELOPMENT OF A MULTI RESIDUE/MULTI MATRIX METHOD FOR PESTICIDE RESIDUES IN AGRICULTURAL PRODUCTS

L. G. M. Th. TUINSTRA, P. VAN DE SPRENG and P. GAIKHORST

State Institute for Quality Control of Agricultural Products (RIKILT-DLO), P.O. Box 230, 6700 AE Wageningen, The Netherlands

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In the Netherlands for the determination of more than 400 pesticides thirteen multi residue methods and about 170 single residue methods are in use. A literature search learns that about 200 pesticides are gaschromatography (GC) amenable, that quantitative extraction procedures exist and these pesticides also give good recovery when gelpermeation (GPC) is used for cleanup of extracts. A strategy is discussed to develop a universal multi pesticide method, especially paying attention to plant material, using a universal very sensitive detection system, e.g. the ion trap detector.

Experimental data obtained with diverging matrices are presented together with some thoughts on on-line GPC-GC combinations, using a temperature programmable injector in the GC system.

KEY WORDS: Gas chromatography, gel permeation chromatography, ion trap detection, pesticides, multi method.

INTRODUCTION

At this moment about 450 pesticides are allowed to be used in the Netherlands. For the majority of these pesticide methods are described[1]. About 170 pesticides can be analysed using so called single residue methods, about 230 pesticides are covered in thirteen multi residue methods. In 1995 a new edition is planned. The monitoring of all pesticide/commodity combinations is far beyond the resources of combined Dutch laboratories, mainly Food Inspection Services.

In the twelve countries of the EC a positive list of pesticides will appear as an annexe to a EC directive. It is estimated that about 700 pesticides will be on this list; meaning that in the Netherlands even more pesticides have to be checked for their presence in food.

In the frame work of a quality programme for agricultural products the Dutch Ministry of Agriculture, Nature Management and Fisheries has funded the development of a general

applicable method, for the time being focussed on GC amenable pesticides. In this publication several thoughts to solve the problem will be mentioned as well as results will be reported.

EXPERIMENTAL

All work is carried out on a Finnigan ITS40, updated with hard- and software into a Magnum and Tracker system. A Varian 3400 GC was equipped with a Finnigan A200S autosampler and a 25 m J&W DB5 capillary, i.d. = 0,25 mm, df = 0,12 µm. Carrier gas is helium, linear velocity about 35 cm/sec, injector volume 2 µl splitless, temperature programming, 5 min at 90°C, than heated up to 300°C, with a mean velocity of 5°/min. Each second a spectrum (4 µscans) was recorded from m/z 60-500 in electron impact mode, starting 4 min after injection.

One of the first aspects in the whole set up of the procedure was to check in how far pesticides, selected from literature for their GC behaviour, indeed could be detected at the needed level (100 pg as minimum identification level). This demand in fact contains two prerequisites. The compound should not fragmentate too much in the source of the ITS40. On the other hand several compounds will only pass through the GC system, when this is in a perfect condition. Too many "active spots" will prevent, that enough pesticide per time unit reaches the ITS source and will therefore not meet the demand, and will not be detectable at all!

Up till now 112 pesticides have been injected at concentrations of 100 pg/µl. From these, 109 indeed, at the time of analysis, met the requested identification level (IDL). It must be emphasized that for a certain number of pesticides this is the situation for that moment. The condition in the GC system, from injector up to transfer line into the ITS source, can change due to injection of dirty sample extracts, leakage of oxygen destroying the GC capillary, etc.

In Table 1 for a given mixture of ten pesticides the coefficient of variation of the determined quantity, using PCB 198 as an internal standard, is given for thirteen injections.

All used pesticides, with exception of coumaphos (azinphos-ethyl and -methyl not tested at that level at that time), give an IDL of 100 pg/µl. It is clear that phorate at these low levels

Table 1 Coefficient of variation for the quantification of several pesticides in a test mixture, using PCB 198 as internal standard.

Pesticide	*Concentration pg/µl in mixture*	*C.V. of quantification %*
Phorate	100	5
Pirimiphos-methyl	150	6
Malathion	200	7
Chlorpyriphos-ethyl	150	5
Chlorfenvinphos	500	8
Methidathion	250	9
Triazophos	400	11
Azinphos-ethyl	400	17
Azinphos-methyl	500	17
Coumaphos	400	25

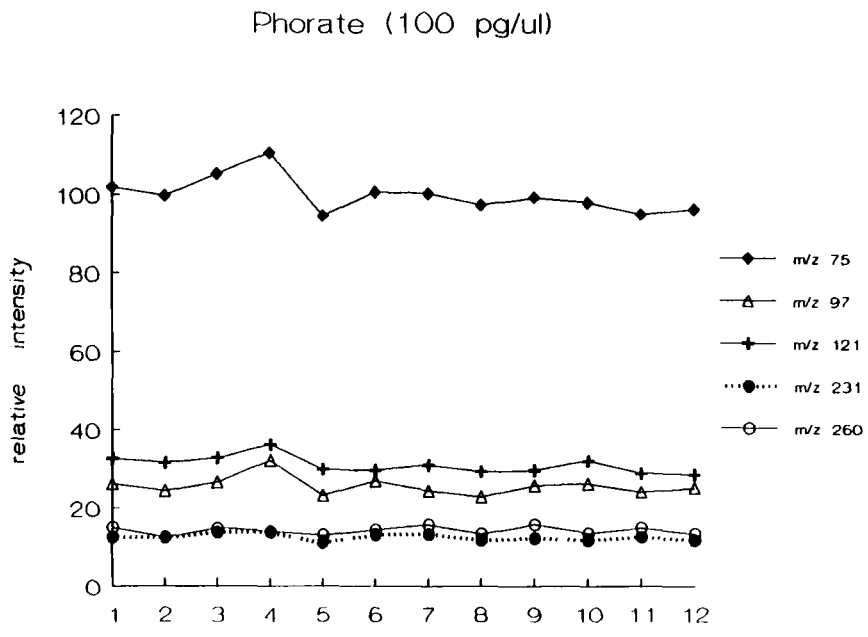

Figure 1 Relative responds of five ions taken from the spectrum of phorate, injected at 100 pg/µl (2 µl injections), in the time (12 hrs).

easily can be identified and quantified. Coumaphos showing poor GC behaviour, gives considerable problems.

Another way of looking at GC-ITS behaviour is shown in Figure 1. in which for several significant ions of phorate the response for each ion in the time is given. To do these exercises for all pesticides is an impossible job.

Identification of compounds can be carried out in several ways. In the beginning of the project all kind of pesticides have been injected to record their MS spectrum and retention time. The spectra are put into the Library File (LF). From this LF a Quantification Calibration File (QCF) is made containing at least a reduced mass spectrum, the retention time (RT), the RT window (e.g. 60 sec.), a fit threshold and a minimum signal/noise ratio.

In case of analysis of unknown samples the retention time of an unknown compound ± halve the retention time window, is used to select only those compounds from the QCF that could have eluted form the GC column in that time period and to see in how far these (reduced) spectra of these selected compounds fit within the complete spectrum of the unknown compound from the sample. If so, a compound is identified. All other spectra in the QCF outside the RT window are not used for the identification of the unknown compound in the sample. Doing so much computer time is saved. Another advantage, over reverse fit, is that when two compounds elute more or less at the same retention time, and therefore produce a mixed spectrum each one will be recognized. In the FIT procedure the appropriate reduced spectra are taken and checked for their fitting into the unknown (mixed) spectrum and than (depending on the set fit threshold) are identified in that (mixed) spectrum.

An example is found in the pesticides pirimiphos-methyl and fenitrothion. Under the used GC conditions the difference in retention time is less than 1 sec. The mass spectra differ considerably. In the reverse fit only the first eluting compound (e.g. fenitrothion) will be identified. In the forward search procedure both.

STRATEGY FOR A METHOD

Separation and detection

In the today's analysis of GC amenable pesticides, columns are connected to different detectors: electron capture, Hall, nitrogen-phosphorus, flame photometric detectors. Several GC's are needed. In recent years bench top mass spectrometers became popular and relatively cheap. End 1990 with Finnigans ion trap ITS40 full spectrum could be obtained with about 100 pg of compound[2]. At that time quadropoles could offer this sensitivity only in single ion detection mode[3] so that analyses could only be carried out for target compounds. When too many ions had to be measured, sensitivity decreased dramatically. So an ITS40 system was, at that time, a first choice universal detection system for "volatile" pesticides. In gas chromatographic pesticide residue analysis the use of capillary columns is nowadays standard.

In literature retention behaviour of pesticides on capillaries is described extensively, e.g. in reference 4.

Extraction and clean-up

In literature many methods exist for the analysis of pesticides. Very interesting and popular are the methods of Specht and Tilke[5] on the one hand and the method of Luke[6] on the other hand. Both methods use acetone as extractant. In the Specht method pesticides are extracted from the acetone with dichloromethane by macerating. The lower dichloromethane layer contains the pesticides. In the Luke method the acetone extract is extracted with a mixture of petroleum ether (PE) and dichloromethane, so that the pesticides are in the upper PE layer. In both procedures sodium sulphate is used for drying the organic dichloromethane (+PE) phase.

The Luke method can be carried out in one centrifuge bottle as described in reference 7, simplifying the whole sample handling. Of course the method is downscaled. 10–20 g of sample are used for the analyses. Both methods use solvents that can easily be concentrated.

Another very simple method uses only ethylacetate as an extractant. The method extracts the majority of the pesticides allowed in the Netherlands from the matrix as long as enough sodium sulphate is added for drying the sample, and is because of its simplicity, very popular (e.g. reference 8). Very often, the (concentrated) extract as such is automatically injected into the GC, equipped with a specific detector. Offcourse special attention should be given to the glass liner of the injector. In the Luke method[6] no special clean-up at all is carried out as is, very often, also the situation for the ethylacetate method[8]. Specht[5] used gel permation

chromatography on Bio Beads SX3 as clean-up method with ethylacetate and cyclohexane as eluents.

Also described by Specht[5] is the use of mini silica columns for a final clean-up of the GPC fraction, containing the pesticides. Today several manufacturers produce automated equipment for solid phase extraction. Application of the ASPEC (from Gilson) in an on-line coupling to a GC system for analysis of pesticides is in use for routine analysis[9].

From these three publications no clear conclusion can be drawn if an extra clean-up step is a must. GPC is an elegant technique, highly automated and compatible with all kind of eluents[5,10–13]. The choice of the GPC eluant is of importance. Ethyl acetate is very often one of the components, but has a relative high boiling point and does not form azeotropic mixtures, resulting in a much lower boiling point. Cyclohexane, another very often used component of the GPC eluant gives with acetone an azeotropic mixture (ratio 1:2) with boiling point of 53°C[10]. Especially as the GPC fractions should be concentrated, this lower boiling point can speed up the analysis time and prevent losses of more volatile pesticides.

Data with respect of successful GC and GPC behaviour were put into a data base. From the 450 pesticides allowed in the Netherlands 276 can, without derivatisation, be analysed on a capillary column. From the 450 compounds 269 pesticides give 80% or higher recovery from GPC systems (Bio Beads SX3). Combination of all data learned that at least 209 pesticides will pass through GPC and GC column. Most of these compounds have been tested[5] for extraction efficiency and additional clean-up on silica columns.

It should be noted that this "desk" study learns that 53 pesticides from these 209 in[1] are described as a single residue method (SRM).

Assumptions

In the Dutch pesticide legislation maximum residue limits (MRL) range from 0,01 mg/kg level up to mg/kg level. From an analytical view, the worst situation is an MRL of 0,01 mg/kg, especially when it is felt necessary to use a limit of detection ten times lower than this MRL. Assuming that the ITS40 detection system needs 100 pg to provide an identifiable and quantifiable MS spectrum for the lowest MRL, this means that each gram of sample matrix should contain at least 1 ng of compound.

Practical impact

Focussing attention on plant material, (because probably much more pesticides will pollute plant material than fat material) and referring to extraction procedures according to references 5, 6 and 8, an interesting possibility looms for an on-line GPC-GC-ITS system. A GPC column with an inner diameter of 1 cm easily can handle the extract of 10 g plant sample. The pesticide containing fraction is about 12 ml. In the light of the above assumptions one can now proceed differently. One needs to use only $1/100^{th}$ part of the collected fraction; either by injecting 2µl in the GC-ITS system after concentration to 200µl or to inject 120µl into the GC-MS of the original fraction.

Another option is to reduce the inner diameter of the GPC column to 2 mm, in which the extract of 400 mg matrix is injected. The GPC fraction containing the pesticides is now about 400µl[14]. Also this volume can be injected into the GC-ITS, using a loop type interface[15,9], without problems.

Big volumes can also be injected into the GC using a PTV (Programmable Temperature Vaporiser). The solvent evaporates in the packed injector liner, kept at low temperatures, and is split of. Pesticides and coextractants are withheld in the packing (e.g. glasswool). Then the temperature of the injector is increased so that volatile compounds move into the "cold" GC column. After trapping, a normal temperature program elutes the pesticides into the detector. During the recent 15th International Symposium on Capillary Chromatography in Riva del Garda, Italy, several papers paid attention to this type of PTV application.

RESULTS

Several experiments with matrices and several groups of pesticides have been carried out to test the assumptions made in the beginning of the project.

In one experiment the matrices tomato, cucumber, cauliflower, capsicum, chicory, potatoes, apples, endive and wheat where spiked with seventeen N-containing pesticides at the 0,25 mg/kg level. The procedure of Specht[5] was used for extraction and clean-up. In Table 2 also the lowest MRL for each pesticides in one of the above mentioned matrices is given. Even when recoveries were sometimes lower than expected (probably due to too fast evaporation of the GPC fraction) the quality of the obtained spectra was that good that levels at lowest MRL's indicated in Table 2 should be detected without problems.

In Table 3 results for the determination of eleven pesticides in real salad and spinach samples (n=39) are given obtained with the Specht[5] procedure, together with the MRL for the pesticides in the matrices and the estimated IDL in mg/kg. Not one of the found pesticides exceeded the MRL, though iprodione was found nine times, vinchlozolin and permethrin both four times above the IDL for the appropriate matrix.

In Figure 2 a blown up chromatogram plot of a salad sample is shown, together with the corresponding spectrum and the result of the library search, showing the presence of vinchlozolin. In Figure 3 in the same sample the presence of iprodione as proven.

Table 2 Lowest MRL (mg/kg) for several pesticides in different vegetables (see text)

Alachlor	0.02	Metribuzin	0.1
Benodanil	0.05	Nitrofen	0.01
Biphenox	0.05	Nitrothal-isopropyl	0.05
Bromacil	0.05	Pendimethalin	0.05
Chlorpropham	0.05	Pirimicarb	0.5
Crufomate	0.05	Triadimefon	0.05
Diallate	0.1	Triallate	0.05
Fenamirol	0.1	Trifluralin	0.01
Imazalil	0.05		

Table 3 Contents of several pesticides in salad and spinach samples, MRL's and IDL's

Pesticide	*MRL (mg/kg)*		*IDL mg/kg*	*Found range mg/kg*
	Salad	*Spinach*		
Diazinon	0.5	0.5	0.02	-
Dichlofluanide	10.0	5.0	0.1	-
Dichlorphos	0.1	0.1	0.02	-
Heptenophos	0.1	0.1	0.01	-
Iprodion	5.0	5.0	0.05	0,09–0,64
Malathion	3.0	3.0	0.02	-
Mevinphos	0.5	0.5	0.05	-
Permethrin	2.0	2.0	0.05	0,05–0,24
Pirimicarb	1.0	1.0	0.05	-
Propoxur	3.0	3.0	0.05	-
Vinclozolin	5.0	1.0	0.02	0,06–0,35

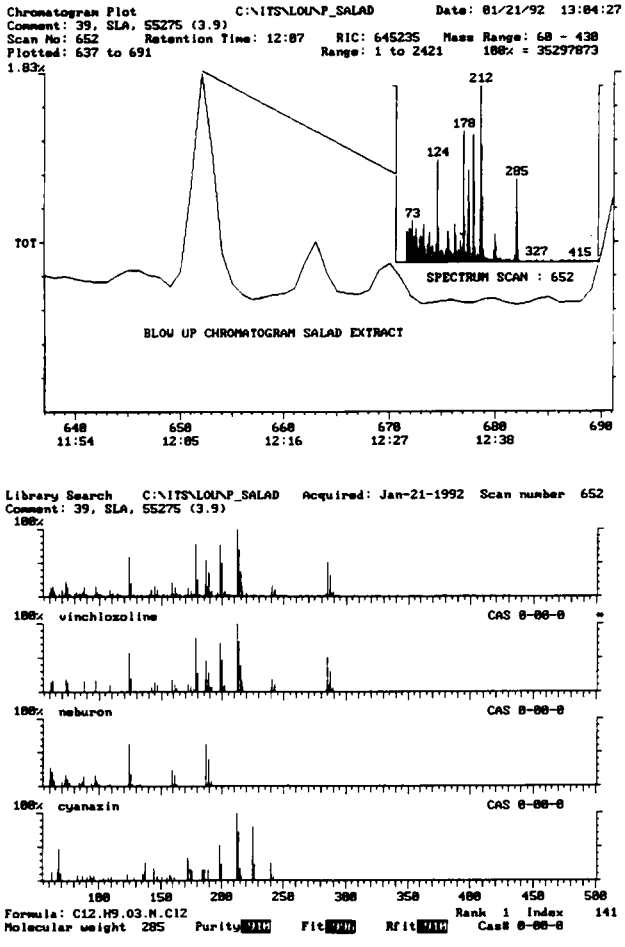

Figure 2 Blown up total ion current chromatogram of a salad extract (#55275), spectrum of a unknown peak (scan 652) and result of library search.

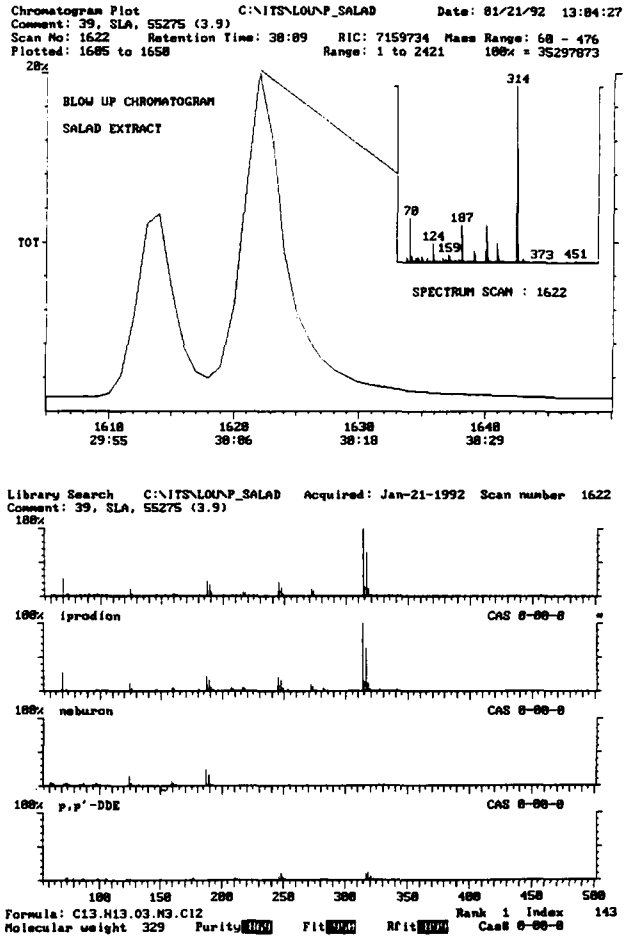

Figure 3 Same as Figure 2, spectrum taken at scan no. 1622.

In table 4 an overview, extracted from a data base containing all relevant information, is given for the pesticides that should be determined in apples and pears in the frame work of a project for consumer organizations: the lowest MRL of a pesticide in one of the two matrices, the possibility of GPC clean-up and GC amenability. The first column gives an internal used identification number of the pesticide standard. The last column, giving the IDL in an absolute way, shows that for all pesticides the 100 pg assumption is realistic.

The apple and pear samples have been extracted as described in[8] and 0,5 ml ethylacetate, containing 5 g sample extract, was injected into a GPC system[10], eluted with acetone/cyclohexane (2:1). The collected fraction was concentrated to 0,5 ml and from this 2 µl was injected into the GC-ITS40 (≈ 20 mg sample).

Matrices mentioned above give after extraction and GPC clean-up[5] a rather clean RIC chromatogram. With onion and probably also with other spicy products, the situation is

Table 4 Determination of pesticides in real apple and pear samples

No.	*Pesticide*	*LMRL*	*GPC possible*	*GC possible*	*IDL pg/μl*
7	Azinphos-methyl	0.5	+	+	100
11	Bromophos	1	+	+	100
10	Bromophos-ethyl	0.5	+	+	100
390	Captafol	0.05	+	+	100
17	Captan	0.1	+	+	100
665	Chlorpyriphos-met	0.02	+	+	100
447	Cypermethrin	0.2	+	+	100
437	Deltamethrin	0.1	+	+	100
89	Dialifos	0.05	+	+	100
42	Diazinon	0.5	+	+	100
55	Dimethoate	1	+	+	100
73	Fenitrothion	0.5	+	+	100
586	Fenpropathrin	0.05	+	+	100
522	Fenvalerate	0.05	+	+	100
82	Formothion	0.05	+	+	100
249	Heptenophos	0.1	+	+	100
252	Iprodione	0.05	+	+	100
102	Lindane	1	+	+	100
31	Malathion	0.5	+	+	100
113	Methidathion	0.02	+	+	100
83	Mevinphos (C+T)	0.1	+	+	100
80	Omethoate	0.2	+	+	100
127	Parathion	0.5	+	+	100
313	Permethrin	1	+	+	100
86	Phosalone	1	+	+	100
85	Phosmet	0.05	+	+	100
84	Phosphamidone	0.2	+	+	100
352	Pirimiphos-methyl	0.02	+	+	100
133	Quintozene	0.01	+	+	100
521	Tolyfluanid	0.1	+	+	100
191	Triazophos	0.01	+	+	100
186	Trichlorfon	0.05	+	+	100
192	Vamidothion	0.05	+	+	100
250	Vinclozolin	0.02	+	+	100

different (Figure 4). At lower retention times many coextracted compounds are present in the RIC chromatogram. Recovery experiments, meeting a FIT threshold of at least 850 for the identification of spiked pesticides, resulted in no recovery at all for early eluting pesticides. When the FIT threshold was reduced to 500 an identification (and quantification) was possible and resulted in acceptable recoveries. Practically this means that with too high setting of the threshold many false negatives can occur. The threshold set too low, can result in many false positives. If in the latter situation the goal is the controlling of MRL's, no further action is necessary as long as the quantitative result is far below the MRL. Otherwise, a careful manual comparison of the unknown spectrum with the standard spectrum is unavoidable.

Though GPC separates larger molecules from the pesticides not all compounds injected into the GPC behave according to theory. For instance colouring materials, being larger

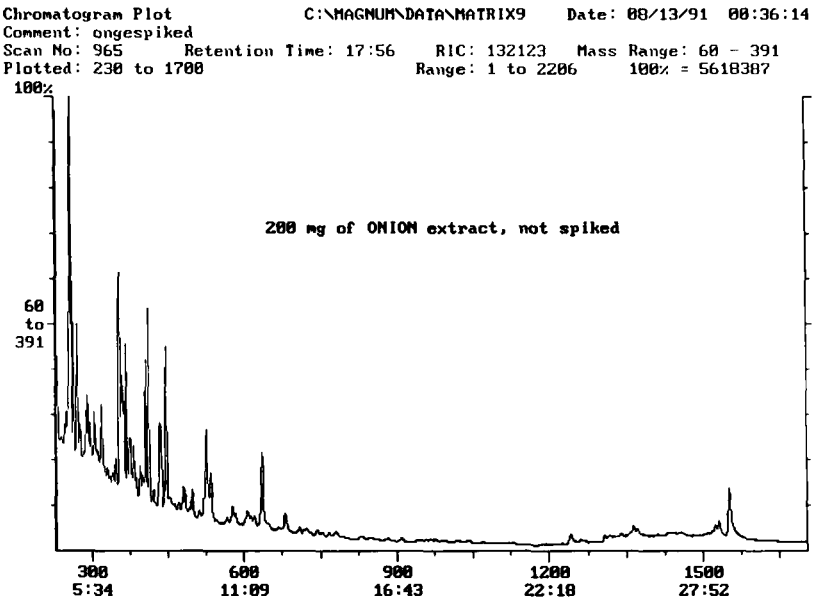

Figure 4 RIC chromatogram of an onion extract obtained according to ref. 5.

molecules, show not a GPC behaviour but adsorb to the Bio Beads material and elute slowly from the GPC column and will contaminate next samples. Of course smaller molecules (e.g. triglycerides) can also elute within the pesticide fraction. In the experiments up till now no problems of strong changes in retention time, poor peak shape have been observed. Of course the glass liner of the injector was regularly cleaned (each 3 days). The GPC column is in use already for years, without problems.

Off line, but also on-line, injection of the pesticide containing GPC fraction into the GC is a (potential) contamination source of the liner and the GC column. Protection of the GC column can be realized by use of a retention gap. An interesting new technique seems to be PTV injection also for big volumes (up to 1 ml) of pesticide solutions[16,17].

CONCLUSION

In several orientating practical sample investigations the basic concept is proven. The use of a "best choice" extraction solvent, combined with a universal clean-up on GPC, followed by separation on a capillary column and ion trap detection offers a practical quantification technique for the determination of many GC amenable pesticides in one analytical run. In future several multi residue methods and many single residue methods can be combined in a single one multi method, saving a lot of time and money or offering the possibility to increase the number of analysis.

However before that point is reached the behaviour of appropriate pesticides have to be tested, also in combination with diverging matrices. A test mixture containing pesticides, difficult to analyse from a point of view of their GC behaviour or their MS spectrum (fragmentation), should be developed for day to day testing of the whole detection system.

On line coupling with small diameter GPC columns is possible.

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