A Method Used to Screen for 567 Pesticides and Suspected Endocrine Disrupters

Application

Gas Chromatography

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Abstract

A gas chromatographic (GC) method has been developed that can be used to screen for 567 pesticides and suspected endocrine disrupters. In principle, it can be used to screen for any GC-amenable pesticide, metabolite, or endocrine disrupter. The method relies on a technique called retention time locking (RTL). RTL is a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with the same stationary phase, nominal phase ratio, and diameter are used. Because RTL increases retention time precision and predictability, raw retention times can be used as a more reliable indicator of compound identity. The chromatographer first locks the GC method so that all retention times match those listed in a 567-compound pesticide and endocrine disrupter retention time table. After analyzing a sample by GC with atomic emission detection (GC-AED), the analyst enters a peak's retention time and known elemental content (presence or absence of heteroatoms) into a dialog box. If element-selective detectors are used, detector response can be entered in addition to or in place of GC-AED data. The software then searches the pesticide table for those compounds that elute at the correct retention time and have the right elemental content or detector response. Most often, the software finds just one compound that meets these criteria, and rarely does it find more than three. Confirmation is performed by GC with mass spectral detection (GC-MS) or by calculation of elemental ratios using GC-AED data. With retention time locking, pesticides have the same retention time on all GC systems; this makes GC-MS confirmation much easier because the analyte's retention time is already known.

Key Words

Pesticides, endocrine disrupters, gas chromatography, retention time locking, RTL

Introduction

The Pesticide Manual[1] lists 759 compounds and biological agents that are used currently as active ingredients in various pesticide formulations. Many compounds, though no longer used, still persist in the environment. For the protection of human health and the environment, acceptable limits in food and water have been set by governmental bureaus such as the United States Environmental Protection Agency (USEPA) and the Codex Alimentarius Commission.[2] Numerous methods have been developed to screen for pesticide contamination in food[3–7] and the environment[8–10] to ensure that these standards are met.

Certain pesticides and other synthetic chemicals have been suspected of behaving as pseudo hormones, disrupting normal functions of the endocrine system in wildlife and humans. Maladies such as birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence have been blamed on exposure to endocrine disrupters.[11] The 1996 publication of *Our Stolen Future*, a book by Colborn,

Dumanoski, and Myers,[11] brought these concerns to the attention of the public. Recently passed legislation in the U.S. calls for more testing of suspected endocrine disrupters and monitoring of them in food[12] and water[13] supplies. To facilitate more research into the endocrine disrupter issue, methods are needed to detect suspected compounds at trace levels.

Because so many pesticides are in use, it is usually impractical to screen for large numbers of them individually and, therefore, multiresidue methods are preferred. Most laboratories that analyze for pesticides in food or the environment screen for only a few dozen compounds because it is often very difficult to screen for more. Recently however, methods have been developed using gas chromatography with mass spectral detection (GC-MS), that can screen for more than 200[5] or even 300[6] pesticide residues.

Still, there is no universal method to analyze for all GC-amenable pesticides. While GC-MS methods are gaining in popularity, there are still some limitations. When methods employ selected ion monitoring (SIM) or tandem mass spectrometry (MS-MS), method development is more tedious and any shift in GC retention times requires that individual analyte retention time windows be shifted accordingly. These methods are only capable of detecting compounds on the target list; there are still hundreds of pesticides, metabolites, and suspected endocrine disrupters that could be missed. On the other hand, methods based on scanning GC-MS alone may require more sample cleanup to avoid interferences from co-extracted indigenous compounds. Typically, these methods do not screen for many pesticide metabolites, endocrine disrupters, or other environmental contaminants. A method that could be used to screen for endocrine disrupters and almost all of the volatile pesticides and metabolites would offer a better means of monitoring the food supply and the environment.

This paper describes a universal method that, in principle, could be used to screen for any pesticide, metabolite, or endocrine disrupter that can elute from a gas chromatograph. The screening procedure relies on a new gas chromatographic technique called retention time locking (RTL)[14–16] with database searching based on retention time and elemental content or detector response. This technique is used to narrow an analyte's identity to a few possibilities. Confirmation is performed by GC-MS or by calculation of a compound's elemental ratio using GC with atomic emission detection (GC-AED).

Experimental

Standards and Extracts

Pesticide standards used to develop the retention time table were obtained from Chem Service (West Chester, PA, USA), Promochem Ltd (Welwyn Garden City, Hertfordshire, England), Dr. Ehrenstorfer (Augsburg, Germany), Hayashi Pure Chemical Industries, Ltd (Osaka, Japan), Wako Pure Chemical Industries, Ltd (Osaka, Japan), and GL Sciences Inc (Tokyo, Japan).

Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee, FL, USA). Samples were extracted with acetonitrile followed by solid-phase extraction (SPE) using a C-18 cartridge. Extracts intended for analysis by halogen-selective detectors were also subjected to floracil SPE.

Pesticide Retention Time Table

The table containing GC and GC-MS retention times for 567 pesticides, metabolites, and suspected endocrine disrupters was obtained from Agilent Technologies, Wilmington, DE, USA (G2081AA).

Instrumentation

Table 1 lists the instrumentation and chromatographic conditions used for GC-AED screening and GC-MS confirmation.

Software for Method Translation

Software for use in translating the normal GC method to one that runs three times faster was obtained from Agilent Technologies,Wilmington, DE, USA.[17]

Results and Discussion

Retention Time Locking

Key to the development of this method is a new concept in gas chromatography called retention time locking (RTL).[14–16] Agilent RTL software allows the chromatographer to match analyte retention times from run to run, independent of the GC system, detector, or manufacturing variations in column dimensions. The only requirement is that the columns used have the same stationary phase and the same nominal diameter and phase ratio. For example, with RTL it is possible to match analyte retention times on a GC-AED and a GC-MS even though the MS operates under vacuum and the AED operates at 1.5 psi above ambient pressure. The

procedure also compensates for differences in GC column length resulting from variations in manufacturing or from column cutting required during routine maintenance.

RTL is accomplished by adjusting the GC column head pressure until a given analyte, such as an internal standard, has the desired retention time. When this is done, all other analytes in the chromatogram will have the correct retention times as well. Software has been developed that can be used to determine the column head pressure that will lock the retention times correctly after one or two "scouting" runs.

With RTL, it is possible to measure pesticide retention times using a given GC method, and then reproduce those retention times in subsequent runs on the same or different instruments. With this increased retention time precision and predictability, retention times become a far more useful indicator of analyte identity. For many years, relative retention times[3,6] or retention indices[18,19] have been used to identify compounds. These techniques were developed to compensate for the fact that retention times were not predictable from day to day, column to column, or instrument to instrument. With the increased retention time precision of the Agilent 6890 GC and RTL, it seemed that raw retention times could be used for compound identification instead of retention indices. The chromatographer could simply scan a table of pesticide retention times, eliminating all possibilities but those with close elution times under the same locked GC conditions.

Table 1. Instrumentation and Conditions of Analysis

Agilent GC-AED System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Atomic emission detector	G2350A atomic emission detector
Computer for data acquisition and analysis	HP Vectra XM Series 4 5/150
Software	G2360AA GC-AED software running on Microsoft® Windows™ 3.11
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
GC inlet	Split/splitless, 250 °C or 260 °C
Inlet liner	Single-tapered deactivated (part no. 5181-3316) with 2-cm deactivated glass wool plug centered ~3 cm from the top
Injection volumes	3–5 µL splitless when running method at 3× speed; 2–3 µL splitless at 1× speed
Inlet pressure (splitless)*	87.5 psi constant pressure for method at 3× speed; 27.6 psi constant pressure for 1× speed
Inlet pressure program (pulsed splitless)*	60 psi (2.01 min), 10 psi/min to 27.9 psi (hold)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C (0 min), 8 °C/min to 280 °C (10 min)
AED transfer line temperature	290 °C
AED cavity temperature	320 °C
AED elements and wavelengths (nm)	Group 1: Cl 479, Br 478 Group 2: C 193, S 181, N 174 Group 3: P 178 Group 4: F 690 (optional)
Agilent GC-MS System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Mass selective detector	5973 MSD
Computer for data acquisition and analysis	HP Vectra XU 6/200
Software	G1701AA Version A.03.00 running on Microsoft® Windows® 95
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
Inlet	Split/splitless, 250 °C
Inlet liner	Single-tapered deactivated with small amount of glass wool at the bottom (part no. 5062-3587)
Injection volume	2 µL
Inlet pressure*	15.5 psi (constant pressure)
Oven temperature program	Same as GC-AED
MSD parameters	
Acquisition mode	Scan (35–550 amu)
EM voltage	200 rel
Solvent delay	3.20 min
Threshold	150
Scans/sec	2.86
Temperatures	Transfer line = 280 °C, MS quad = 150 °C, MS source = 230 °C

*The column head pressures shown are typical values. Exact values were determined as part of the retention time locking procedure.

Pesticides almost always contain heteroatoms and often have several in a single molecule. The most frequently encountered heteroatoms are O, P, S, N, Cl, Br, and F. GC with atomic emission detection (GC-AED) has been shown to be a useful tool for pesticide screening because it is selective for all of the elements found in these compounds.[20–22] Thus, GC-AED screening provides valuable information about the elemental content of an unknown molecule. By including this elemental information along with the retention time, it should be possible to narrow pesticide "hits" to just a few possibilities.

To implement this screening procedure, a table of pesticide and endocrine disrupters retention times had to be created using a suitable method under locked conditions.

GC Method for Pesticide Screening

First, a GC method was needed that could elute hundreds of pesticides and endocrine disrupters in a reasonable time with adequate separation. However, the goal was not to separate every possible analyte in a single GC run. Because the intention was to build a table of locked retention times using this method, it had to reproduce these retention times under a variety of conditions. For example, the method needed to accommodate a variety of injection techniques including splitless, pulsed splitless,[23,24] cold splitless using a PTV inlet, and on-column injection which is occasionally used for the more labile pesticides.

The method also needed to perform well with samples dissolved in common solvents such as acetone and methylene chloride. Because a retention gap (or guard column) is sometimes added to protect the analytical column, the method had to be tested to see if it could still be locked with a retention gap installed.

The column chosen for the method was a 30 m × 0.25 mm × 0.25 µm HP-5MS because the same column could be used with any GC-detector combination. In particular, this column was chosen for its low bleed at high temperatures and because its optimum column flow is compatible with GC-MS. The 5% phenyl methyl silicone phase in this column has been widely used for pesticides.

Method translation software[17,25,26] can be used to increase the speed of a method while retaining the same relative retention times. This can be done by translating the method to a column having the same phase ratio but a smaller id or by increasing the flow rate and oven temperature program while using the same column. The final goal was to design a method that could run at three times the normal speed on the 30-m × 0.25-mm × 0.25-µm HP-5MS column or be translated to a 100-µm id column.

After several weeks of method development, the GC oven temperature program shown in figure 1a was chosen because it met all of the development criteria. Chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) was chosen as the locking standard. It is an ideal choice because chlorpyrifos-methyl elutes near the middle of the chromatogram (16.596 minutes), has good peak shape, and can be seen by most element-selective detectors. Because GC-AED requires three runs to generate element-selective chromatograms for C, Br, Cl, N, S, and P, the method was translated to run three times faster using software for method translation.[17,25,26] The faster oven temperature program used by this method requires 6890 GC systems that are configured for fast oven temperature ramping. The method translation software can be used to speed up the method by any desired factor; even 120-V 6890 GCs can run the method two times faster. However, the original method must be used for GC-MS because of the restriction in flow rates into the MSD. Figure 1b lists the threefold (3×) faster GC method.

Pesticide Retention Time Table

Once developed, this method was employed to create a table of locked retention times for the 567 pesticides, metabolites, and suspected endocrine disrupters. Increasing international food trade requires the analysis of pesticides that may be used in the supplying country but not in the recipient country. The goal was to create a table that included pesticides used around the world so pesticide standards were obtained from sources in Europe, Japan, and the USA.

A list of suspected endocrine disrupters was compiled from various lists published on the World Wide Web.[27–31] Many of these compounds are, in fact, pesticides. Most of the GC-amenable endocrine disrupters were analyzed and their retention times appear in the table. However, the 209 polychlorinated biphenyl congeners were not included because their inclusion might actually complicate the identification of organochlorine pesticides.

Standards, diluted to 10 ppm in acetone, were first analyzed by GC-MS using the oven temperature program shown in figure 1a and instrumental conditions listed in table 1. Compound identities were verified by matching their spectra to library entries,[32] by comparison with a published spectral compendium,[33] or by matching spectra to a list of charac-

teristic ions.[6] When reference spectral information was not available, the pesticides were verified by spectral interpretation. Samples were then analyzed on two different 6890 GC-FID instruments under the same locked conditions (chlorpyrifos-methyl retention time = 16.596 minutes). The GC-MS retention time and the average of the two GC-FID retention times were tabulated for each compound along with its molecular formula, molecular weight, and CAS number. In addition to these fields, there are four user-definable columns in table 2 that can be used to add such things as mass spectral information, internal catalog numbers, or comments. Table 2 lists a small portion of the database. It must be noted that all retention time values were created using constant column head pressure. This is because GC-MS retention times are very close to those obtained with other detectors when constant pressure is used. In this mode, GC-MS and GC-FID retention times match within ± 0.1 minute except for three compounds that elute at the very end of the chromatogram. Even in this case, the differences are no more than 0.2 minute. The discrepancy between GC-MS and GC-FID retention times is larger in the constant flow mode.

Pesticide Screening Method

Figure 2 diagrams the pesticide screening method. First, RTL was used to match GC-AED and GC-MS analyte retention times to those listed in the pesticide table. Software for RTL[14–16] was used to determine the

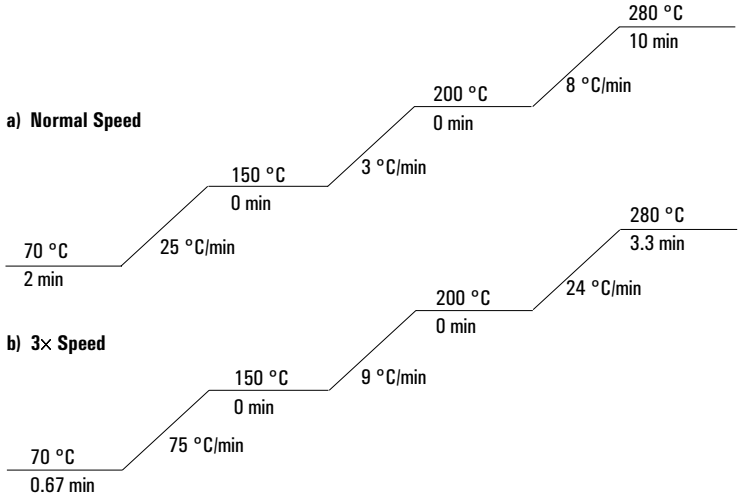

Figure 1. a) GC oven temperature program for the Agilent pesticide method at normal speed. When using this method, chlorpyrifos-methyl must be locked to 16.596 minutes. This method is used by GC-MS and can be used by any other GC system. b) GC oven temperature program for the Agilent pesticide method translated to run three times faster. This method may be used with 6890 GCs configured with any detector except an MSD so long as the GC is configured for fast oven temperature ramping. Chlorpyrifos-methyl must be locked to 5.532 minutes.

Table 2. Small Portion of the Pesticide and Endocrine Disrupter Retention Time Table That Contains 567 Entries. The retention times shown here are for the pesticide method run at normal speed as shown in figure 1a. Chlorpyrifos-methyl was locked to 16.596 minutes (± 0.015 minute for the collection of the tabulated retention time values. The table includes four additional columns for user-defined information.

FID RT	Name	CAS No.	Molecular Formula	MW	MSD RT
16.542	Acetochlor	34256-82-1	C:14,H:20,Cl:1,N:1,O:2,	269.77	16.542
16.549	Fuberidazole	3878-19-1	C:12,H:8,N:2,O:1,	196.21	16.549
16.583	Methyl parathion	298-00-0	C:8,H:10,N:1,O:5,P:1,S:1,	263.20	16.594
16.596	Chlorpyrifos methyl	5598-13-0	C:7,H:7,Cl:3,N:1,O:3,P:1,S:1,	322.53	16.593
16.637	Vinclozolin	50471-44-8	C:12,H:9,Cl:2,N:1,O:3,	286.11	16.630
16.650	Plifenat	21757-82-4	C:10,H:7,Cl:5,O:2,	336.43	16.641
16.689	Terbucarb	001918-11-2	C:17,H:27,N:1,O:2,	277.41	16.686
16.730	Chloranocryl	2164-09-2	C:10,H:9,Cl:2,N:1,O:1,	230.09	16.736
16.752	3-Hydroxycarbofuran	16655-82-6	C:12,H:15,N:1,O:4,	237.26	16.741
16.773	Heptachlor	76-44-8	C:10,H:5,Cl:7,	373.32	16.796
16.800	Carbaryl	63-25-2	C:12,H:11,N:1,O:2,	201.22	16.806

column head pressure needed to produce a retention time of 16.596 minutes for chlorpyrifos-methyl. When analyzing samples by GC-AED, the method was usually run at 3× speed and chlorpyrifos-methyl was locked to 5.532 minutes.

Figure 3 shows the RTL software screen that is used to develop the retention time calibration. To accomplish this for the pesticide method, one should install the 30 m × 0.25 mm × 0.25 µm HP-5MS column (part no. 19091S-433) and set the column head pressure to one of the appropriate nominal values as shown below, making sure to use the constant pressure mode.

- 26 psi for atmospheric pressure detectors run at normal speed (eg, NPD, FPD)
- 16 psi for GC-MSD operated at normal speed
- 27.5 psi for GC-AED operated at normal speed
- 88 psi for GC-AED operated at 3× speed

To prepare a calibration table similar to the one shown in figure 3, the chromatographer must make five analyses of chlorpyrifos-methyl at the following column head pressures: the nominal pressure, the nominal pressure + 20%, the nominal pressure + 10%, the nominal pressure – 10%, and the nominal pressure – 20%. Because of the first run affect, it is usually wise to make one or two blank runs before performing the five calibration runs. The five pressures and the chlorpyrifos-methyl retention times are entered into the table provided by the RTL software. This calibration table stays with the method and can be used to lock, or re-lock, the GC

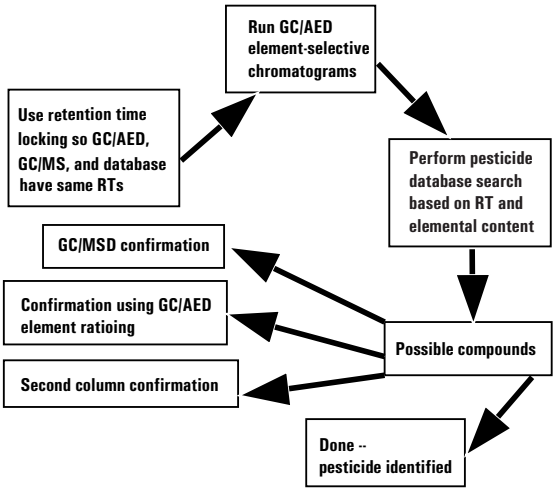

Figure 2. Diagram of the screening method that uses retention time locking and retention time table searching to identify pesticides and suspected endocrine disrupters.

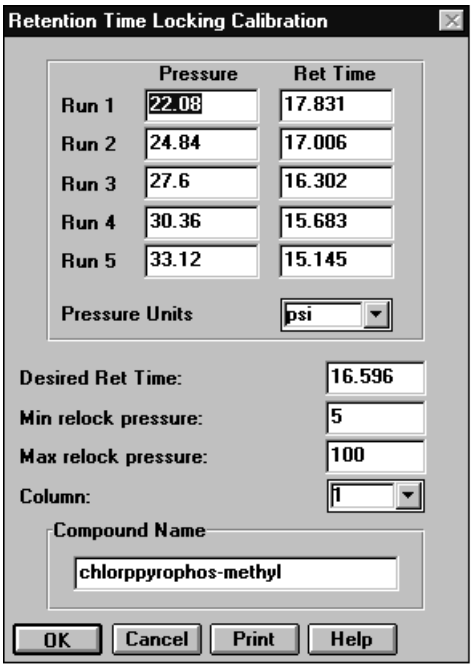

Figure 3. RTL software screen showing typical retention time locking calibration data for the pesticide method run at normal speed using a GC detector that operates at atmospheric pressure.

method as long as that method is used. That is, the five calibration runs only need to be made once for a given method.

The software screen for locking the GC method is shown in figure 4. To lock the method, one enters the retention time of chlorpyrifos-methyl and clicks on the "Calc new pressure" button. The RTL software calculates the pressure needed to lock the chlorpyrifos-methyl peak at the desired retention time. By clicking on the "Update current 6890 Method" button, this value is entered automatically into the method.

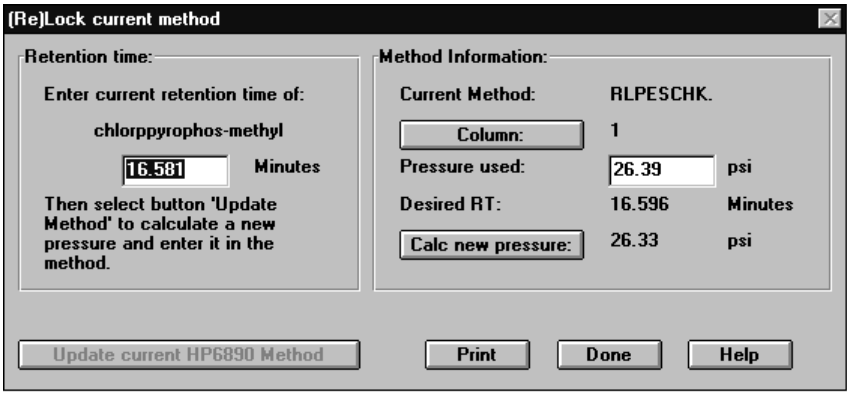

Figure 4. RTL software screen used to calculate the column head pressure needed to lock or re-lock a method. In this case, the chlorpyrifos-methyl retention time was 16.581 minutes and the pressure needed to re-lock the method was calculated to be 26.33 psi. By clicking on the "Update current 6890 Method," button, the new pressure is entered automatically into the GC method.

One can use Agilent's software for method translation[17] to convert the method to other speeds (eg, 1.9×) and determine the nominal column head pressure required. If this is done, the pesticide table must be exported to a spreadsheet program where the analyte retention times can be divided by the appropriate factor (1.9 in this case). This new table can then be imported back into the ChemStation for use with the new method.

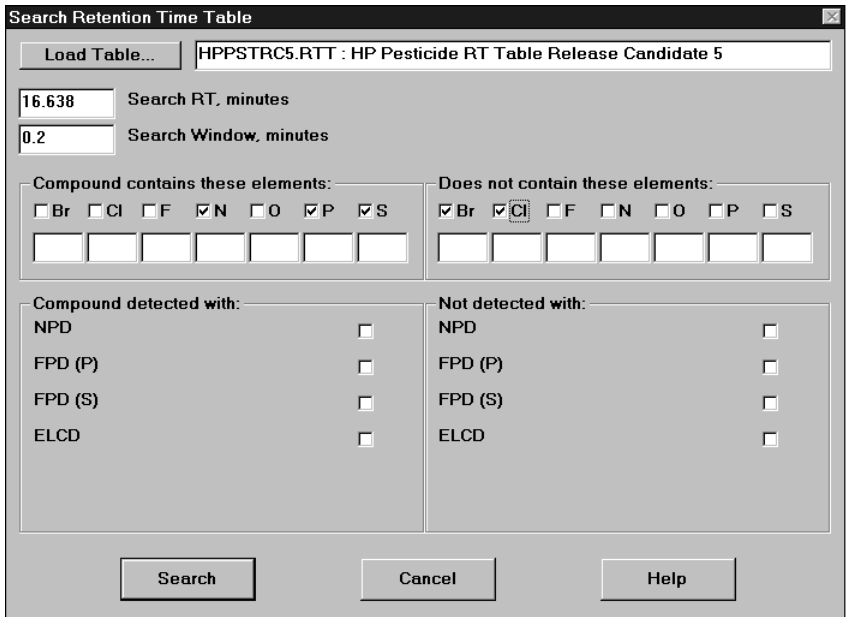

Figure 5. RTL software screen used to search a retention time table on the basis of retention time and known elemental content. In this case, the software will search the Agilent pesticide table at 16.638 ± 0.1 minutes for compounds that contain N, P, and S but do not contain Br or Cl. If element-selective detectors (such as the NPD) are used, this information can be provided to the search routine. Up to six different element-selective detectors can be configured as shown for NPD, FPD (P), FPD (S), and ELCD.

After locking the method to the table, GC-AED element-selective chromatograms were obtained for C, Cl, Br, N, S, P, and sometimes F. From the GC-AED chromatograms, it was usually possible to determine which heteroatoms were present or absent in the suspected pesticide peak. RTL software was then used to search the database by retention time and elemental content. Figure 5 shows the RTL software screen used for retention time table searching. One can enter the elements known to be present or not present in the GC-AED peak of interest. Up to six other element-selective detectors can be configured for use in the search algorithm. When the presence or absence of a heteroatom is uncertain, nothing is added to the search routine for that element.

One must choose a search time window wide enough to include the correct analyte, but narrow enough to eliminate as many extraneous "hits" as possible. Experience has shown that the normal speed method requires a search window of 0.2 to 0.3 minute. The 3× speed method can use a search window of 0.1 minute. If the heteroatom content is known for a peak, retention time table searching

with these search windows most often finds just one pesticide and rarely finds more than three possibilities.

Confirmation is usually done by GC-MS under locked conditions so that all GC-MS retention times match the values listed in the pesticide retention time table. This was found to be of enormous benefit. Prior to GC-MS confirmation, the analyst already knows which pesticides to look for and their expected retention times. Alternatively, when there is adequate signal to quantitate the analyte in multiple AED element-selective chromatograms, it is often possible to confirm a pesticide's identity simply by calculating its heteroatom ratio. GC-AED software for element ratioing facilitates this procedure.

Analysis of a Green Onion Extract

Numerous samples of fruit and vegetable extracts have been analyzed using this methodology. The results for a green onion extract illustrate the versatility and potential of this method.

Green onion extracts are usually very dirty and contain a large number of co-extracted sulfur compounds that can obscure sulfur-containing pesticides. The onion chromatograms shown in figure 6 were run under locked conditions at 2× speed in Tallahassee, Florida, by the Department of Food and Agriculture using a 5890 SERIES II/5921A GC-AED system. Retention time searching indicated that folpet was present in the sample, but it could not be confirmed at the time. The same sample was sent to the Agilent Technologies Little Falls Site in Wilmington, DE, where it was analyzed by scanning GC-MS using an 6890/5973 system. As shown in figure 7, folpet was easily confirmed at the expected retention time. In addition, the pesticides trichlorophenol, chlorothalonil, propoxur, and prochloraz were identified. Searching the Cl peak at about 6 minutes gave no pesticide hits. However, GC-MS suggested the presence of a trichloronaphthalene isomer at the corresponding retention time in the GC-MS chromatogram (about 12 minutes because the GC-MS was operated at normal speed). Though not a pesticide, trichloronaphthalene is considered to be a hazardous compound that should not be in food.

The same green onion sample was then analyzed by the newer model GC-AED system (6890/ G2350A) at 3× speed (figure 8). Several more pesticides were identified by searching the pesticide/ endocrine disrupter table using a 0.1-minute retention time window. Table 3 lists the pesticide hits that were obtained for each retention time search using the available GC-AED data. Sulfur was not included in any of the searches because onion extracts have such a high sulfur background.

Confirmation by GC-MS was much easier because the GC-MS retention time for each pesticide hit was printed out with the RT search report. Thus, the retention times and probable identities of each pesticide were already known before the GC-MS analysis was run. As is shown in figure 7 for folpet, one can simply extract the ions characteristic for each pesticide hit and look in the extracted ion chromatogram at the expected retention time.

Quantitative Analysis

The Agilent pesticide screening method is a qualitative tool to identify any of the 567 pesticides and endocrine disrupters listed in the retention time table. This, of course, is the first step in any pesticide screening method. Quantitative analysis can be performed in one of two ways.

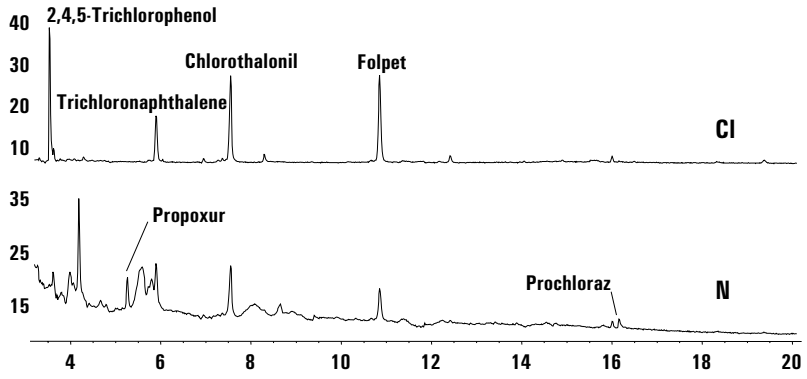

Figure 6. Cl- and N-selective chromatograms of a green onion extract from an 5890/5921A GC-AED system. The analysis was performed at 2× speed under locked conditions in Tallahassee, Florida, by the Department of Agriculture and Consumer Services. In addition to folpet, trichlorophenol, propoxur, and prochloraz were identified by retention time table searching and confirmed by GC-MS at their expected retention times. There were no hits for the Cl peak at about 6 minutes, which was identified by GC-MS as a trichloronaphthalene isomer.

The traditional method is to inject standards into the GC, GC-AED, or GC-MS system to determine response factors from which quantitative results are calculated by the ChemStation software. However, because the GC-AED elemental response is almost independent of molecular structure, compound-independent calibration (CIC) can be used to quantitate all of the pesticides and endocrine disrupters that are found. For example, one could spike chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) at a known concentration into each pesticide extract and obtain element-specific calibration curves for Cl, N, P, and S. These curves could then be used to calibrate for any other compound containing one or more of these elements. Because the GC-AED is quite stable, external standard CIC often works just as well. The GC-AED software facilitates CIC. Unfortunately, this procedure determines the amount of a compound that reaches the AED and does not compensate for losses due to decomposition or adsorption in the inlet or column.

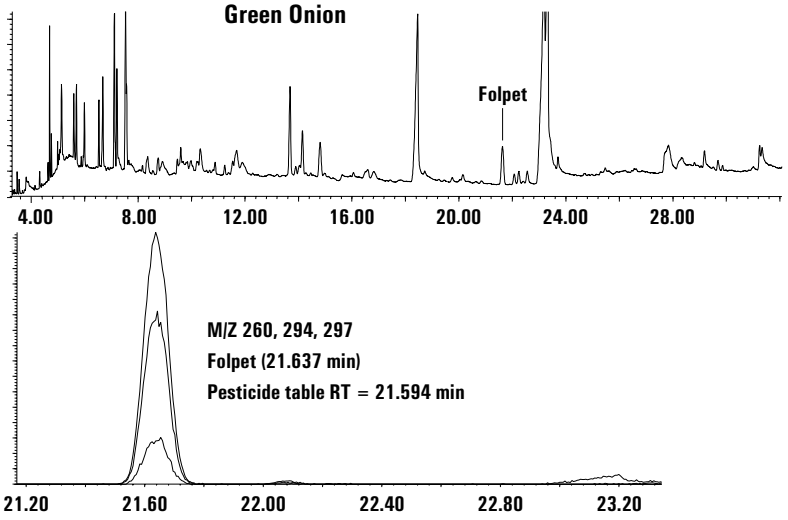

Figure 7. Confirmation of folpet in a green onion extract. The tabulated GC-MS retention time is 21.594 minutes, and folpet was detected in this sample at 21.637 minutes by simply extracting its characteristic ions.

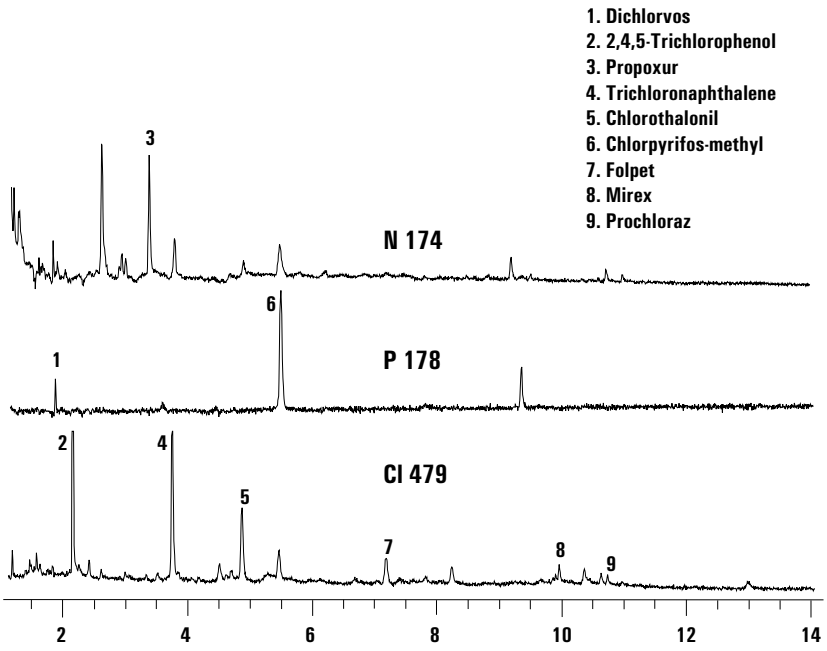

Figure 8. Element-selective chromatograms obtained for the same green onion extract shown in figure 6. These chromatograms were obtained at 3× speed using an 6890/G2350A GC-AED system.

Conclusions

Most screening procedures in use today are capable of finding only a fraction of the pesticides that are registered around the world. This new method has the capability of screening for virtually any volatile pesticide, metabolite, or endocrine disrupter. Although confirmation is usually required, GC-MS analysis is made much easier and more reliable because the pesticide's retention time and probable identity are already known.

While GC-AED is an ideal tool for element-selective pesticide screening,[20–22] many laboratories rely on a combination of other selective detectors. It is still possible to apply this method if each GC system runs the Agilent pesticide method under the same locked conditions. Any combination of GC-AED and/or element-selective detector response data can be entered into the RTL searching software.

When combined with RTL and retention time searching, GC-AED and GC-MS provide the most comprehensive and reliable screening method available for pesticides, metabolites, and suspected endocrine disrupters. Unlike most target compound methods in use today, this procedure has a good chance of finding and identifying unexpected or unknown pesticides, even in complex food extracts. RTL software makes it easy to add more compounds to the method, simply by determining their retention times under the same locked conditions.

Retention time locking with database searching could easily be applied to similar types of analyses. For example, one might use the procedure to identify polychlorinated biphenyls, polynuclear aromatics, drugs of abuse, or flavor and fragrance compounds.

Table 3. Green onion pesticide "hits" obtained by searching the 567-compound pesticide/endocrine disrupter RT table using a 0.1-minute RT window and element-selective GC-AED data. Compounds confirmed by GC-MS are shown for comparison. The GC/MS (figure 7) and GC-AED (figure 8) chromatograms were obtained at normal and 3X speeds, respectively. Sulfur peaks were not used to narrow the search because of the high background of sulfur-containing compounds in the onion extract.

GC-AED RT	RT Search Hits	Confirmed by GC-MS
1.933	Dichlorvos	Dichlorvos
2.281	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol	2,4,5-Trichlorophenol
3.440	Fenobucarb Propoxur 4,6-Dinitro-o-cresol	Propoxur
3.854	No pesticide hits	Trichloronaphthalene isomer
4.955	Terbacil Chlorothalonil	Chlorothalonil
5.538	Chlorpyrifos-methyl	Chlorpyrifos-methyl
7.232	Folpet Chlorbenside	Folpet
9.965	Mirex	Mirex
10.588	Prochloraz	Prochloraz

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References

1. C. Tomlin (Ed.), *The Pesticide Manual*, Eleventh Edition, British Crop Protection Council, Farnham, Surry, UK, 1997.
2. R. W. Miller, *This is Codex Alimentarius*, Secretariat of the Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome.
3. B. M. McMahon and N. F. Hardin (Eds.), *Pesticide Analytical Manual*, Vol. I, Third Edition, U.S. Food and Drug Administration, Washington, D.C., 1994.
4. S. M. Lee, M. L. Papathakis, H.-M. C. Feng, G. G. Hunter, and J. E. Carr, *Fresenius' J Anal Chem* **339**, 376–383, 1991.
5. J. Fillion, R. Hindle, M. Lacroix, and J. Selwyn, *J AOAC Int* **78**, 1252–1266, 1995.
6. Working Group on Development and Improvement of Residue-analytical Methods, *Analytical Methods for Pesticide Residues in Foodstuffs*, General Inspectorate for Health Protection, Ministry of Public Health, Welfare and Sport, The Netherlands, 1996.
7. M. A. Luke, J. E. Froberg, G. M. Doose, and H. T. Masumoto, *J Assoc Off Anal Chem* **64**, 1187–1195, 1981.
8. H.-J. Stan (Ed.), *Analysis of Pesticides in Ground and Surface Water II*, Springer-Verlag, Berlin, Germany, 1995.
9. R. E. Wagner, W. Kotas, and G. A. Yogis (Eds.), *Guide to Environmental Analytical Methods*, 2nd Edition Genium, Schenectady, NY, 1994.
10. U.S. Environmental Protection Agency, *Test Methods for Evaluating Solid Waste*, SW-846, Draft Method 8085: Pesticides by GC-AED.

11. T. Colborn, D. Dumanoski, and J. P. Myers, *Our Stolen Future* Penguin, New York, NY, 1996.

12. Food Quality Protection Act of 1996, Public Law 104-170, Congressional Record, pp. H8127–H8141.

13. Safe Drinking Water Act Amendments of 1996, Public Law 104-182, Congressional Record pp. H9680–H9700.

14. V. Giarrocco, B. Quimby, and M. Klee, Retention Time Locking: Concepts and Applications, Application Note 228-392, Publication (23) 5966-2469E, December 1997.

15. P. L. Wylie and B. D. Quimby, Abstracts of the 1st European Pesticide Residue Workshop, Alkmaar, The Netherlands,| Paper # O-023, June 10–12, 1996.

16. M. Klee and T. Sullivan, Environ. Testing and Analysis, pp. 16–17 and 44–45, March/April 1998.

17. Hewlett Packard Company, *Software for Method Translation*, Available on the World Wide Web at: http://www.chem.agilent.com/cag/servsup/usersoft/main.html.

18. M. L. Lee, F. J. Yang, and K. D. Bartle, *Open Tubular Column Gas Chromatography, Theory and Practice*, Wiley, New York, NY, pp. 217–218, 1984.

19. C. Bicchi, A. D'Amato, and A. Binello, *J High Resol Chromatogr* **19**, 80–84, 1996.

20. S. M. Lee and P. L. Wylie, *J Agric Food Chem* **39**, 2192–2199, 1991.

21. P. L. Wylie and R. Oguchi, *J Chrom* **517**, 131–142, 1990.

22. N. L. Olson, R. Carrell, R. K. Cummings, and R. Rieck, *LC-GC* **12**, 142–154, 1994.

23. P. L. Wylie and K. Uchiyama, *J AOAC Int* **79**, 571–577, 1996.

24. P. L. Wylie, K. L. Klein, M. Q. Thompson, and B. W. Hermann, *J High Resol Chromatog* **15**, 571–577, 1992.

25. W. D. Snyder and L. M. Blumberg, *Proceedings of the Fourteenth International Symposium on Capillary Chromatography*, Baltimore, MD, pp. 28–38, May 25–29, 1992.

26. B. D. Quimby, L. M. Blumberg, M. S. Klee, and P. L. Wylie, "Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking," Application Note 228-401, Publication 5967-5820E, April 1998.

27. http://www.nijs.go.jp/hse/environ/edsubs/substancesnew.html.

28. http://www.wwfcanada.org/ hormone-disruptors.

29. http://www.epa.gov/opptintr/ opptendo/index.htm.

30. http://www.osf-facts.org/basics.chemlist.html.

31. http://www.mst.dk/liste.htm.

32. Hewlett-Packard Company. Mass Spectral Libraries: HP Pest library, Wiley 275 library, NBS75K Library, Palo Alto, CA.

33. Pesticide Reference Spectra, Vol. 1–4, Spectral Service Gmbh, Köln, Germany and Riedel-de Haën AG, Seelze, Germany, 1992.

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