Screening for Pesticides in Food Using the Japanese Positive List Pesticide Method: Benefits of Using GC/MS with Deconvolution Reporting Software and a Retention Time Locked Mass Spectral Database

Application

Food

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Abstract

In 2006, the Japanese Ministry of Health, Labour and Welfare introduced a new system for the regulation of pesticides, feed additives, and veterinary drugs. This "Positive List" system stipulates that only compounds on the approved list can be used in food production and provides the framework for regulation of these compounds. These new regulations have increased the need for analytical methods capable of detecting residues of these chemicals in a wide variety of food products. The majority of the chemicals under regulation are pesticides, and their residues are most often measured by gas or liquid chromatography with mass spectral detection (GC/MS or LC/MS). To address the need for rapid and comprehensive analysis of food samples in the Japanese market, Agilent has introduced a new Japanese Positive List Pesticide Database for use with its Deconvolution Reporting Software (DRS). With this new database and DRS, analysts can screen their GC/MS data files for the 430 GC-amenable pesticides that are being regulated by the Japanese government. The process is fully automated and takes about two minutes per sample.

Introduction

On November 29, 2005, the Japanese Ministry of Health, Labour and Welfare (MHLW) published a "Positive List" system for the regulation of pesticides, feed additives, and veterinary drugs [1, 2]. At that time it published provisional maximum residue limits (MRLs) for 758 chemicals and designated 65 others that would be exempted from regulation. There are 15 substances that must have no detectable residues in food products because of their high risk to humans [3–5]. This "Positive List" system implies that only chemicals listed can be used in agricultural production and any residues must comply with the MRLs set by the Japanese government. Other agricultural chemicals not mentioned have a uniform MRL of 0.01 ppm. This new regulation took effect on May 29, 2006.

Since its introduction, all Japanese agricultural products and imports to Japan have had to comply with the Positive List system. This has led to an increased need for screening agricultural commodities for the pesticides, feed additives, and veterinary drugs on the list.

This application describes a rapid method to screen food extracts for all the GC-amenable pesticides listed in the Japanese Positive List system, together with other pesticides that are monitored by the Japanese Quarantine Stations. In all, the method can screen samples for 430 different pesticide residues. The method uses an Agilent 7890A/5975C GC/MS system running the MHLW GC method with the MSD operating in the scan mode. A new retention time locked (RTL) mass spectral library has been developed specifically for this method. When combined with Agilent's Deconvolution Reporting Software (DRS), it is possible to screen GC/MS data files for all 430 pesticides in about two minutes per sample.

Experimental

The MHLW has published methods for the extraction and analysis of plant- and animal-based foods [6]. Depending upon the target compounds, the extracts are analyzed by GC/MS or LC/MS. The GC/MS method specifies the column phase and dimensions, oven temperature program, inlet temperature, carrier gas, ionization mode and energy, and the ions to be monitored for each compound in a SIM analysis.

The method parameters used here are the same, except that the analysis is usually run in the scan mode so that all ions are monitored for each compound. This facilitates the use of Agilent's DRS, which has significant advantages over other methods.

The instrumentation, software, and instrumental parameters are listed in Table 1.

Samples

The samples analyzed by this method were extracted using the QuEChERS method developed by Lehotay, *et al.* [7, 8].

Table 1. Instrumentation and Parameters for Analyzing Pesticides According to the Japanese Positive List System

Gas Chromatograph	**Agilent 7890A**
Column	Agilent J&W 30-m × 0.25-mm × 0.25-µm DB-5MS (P/N 122-5532)
Retention gap (optional)	5-m × 0.25-mm Siltek Deactivated Fused Silica Tubing [Restek (Bellefonte, PA USA) P/N 10026]
Carrier gas	Helium at a nominal flow rate of 1.0 mL/min with no retention gap. About 1.1 mL/min with 5-m × 0.25-mm retention gap connected to head of GC column. About 1.7 mL/min with 5-m × 0.25-mm retention gap connected to the head of the GC column and a QuickSwap installed and set to a pressure of 5.2 psi.
Retention time locking	Chlorpyrifos-methyl locked to 13.443 min
Oven temperature program	50 °C (1 min), 25 °C/min to 125 °C (0 min), 10 °C/min to 300 °C (10 min)
Inlet	Split/splitless
Inlet temperature	250 °C
Inlet liner	Helix Double Taper Deactivated (P/N 5188-5398)
Automatic Sampler	**Agilent 7683B Series Injector and Tray**
Injection volume	2 µL
Mass Spectrometer	**Agilent 5975C MSD**
Acquisition mode	Scan
Scan range	45 – 550 u
Threshold	0 (or set according to noise level)
Ionization energy	70 eV
Sampling rate	n = 2
Transfer line temperature	280 °C
Solvent delay	3.5 min
Source temperature	230 °C
Quadrupole temperature	150 °C
Tune file	Atune.u
Trace ion detection	On
Software	
GC/MS instrument control	Agilent GC/MS ChemStation (P/N G1701EA, Ver. E.01.00 or higher)
Deconvolution Reporting Software	Agilent Deconvolution Reporting Software (P/N G1716AA, Ver. A.03.00 or higher)
Library searching software	NIST MS Search (Ver. 2.0d or greater) (comes with NIST05 mass spectral library – Agilent P/N G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.64; comes with NIST05 mass spectral library – Agilent P/N G1033A)
MS libraries	NIST05 mass spectral library (Agilent P/N G1033A) Agilent Japanese Positive List Pesticide Library in Agilent and NIST formats (P/N G1675AA)

Results and Discussion

Several years ago Agilent Technologies introduced Retention Time Locking (RTL) for gas chromatography (GC) and GC with mass spectral detection (GC/MS). RTL software makes it possible to reproduce retention times from run to run on any Agilent GC or GC/MS, in any laboratory in the world, as long as the same nominal method and GC column are used [9]. Since any laboratory can reproduce retention times generated in another, it is possible to create mass spectral libraries that contain locked retention times. By locking their method to the published database, users can screen GC/MS files for all of the library's compounds. "Hits" are required to have the correct retention time as well as the correct spectrum, which eliminates many false positives and gives more confidence in compound identifications [9, 10].

More recently, Agilent introduced Deconvolution Reporting Software (DRS) that incorporates mass spectral deconvolution with conventional library searching and quantification. DRS results from a marriage of three different GC/MS software packages: 1) the Agilent GC/MS ChemStation, 2) the National Institute of Standards and Technology (NIST) Mass Spectral Search Program with the NIST05 MS Library, and 3) the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, also from NIST.

DRS performs a normal quantitative analysis for all target compounds that have been calibrated. It then sends the data file to AMDIS, which deconvolutes all the spectra in the total ion chromatogram. (A discussion of deconvolution principles follows.) The deconvoluted spectrum of each peak is then searched against a target compound library – in this case Agilent's Japanese Positive List Pesticide Library, containing 430 compounds. Hits are identified by spectral matching and by comparing their locked retention times to values stored with the library. Because retention times are locked to the library, very narrow retention time windows are used – typically ± 10 seconds around the library's value. For confirmation, the deconvoluted spectra of all hits are compared to the entire NIST05 mass spectral library. The results are summarized in a simple report.

Deconvolution

While a thorough discussion of deconvolution is beyond the scope of this application, the basic principles are illustrated in Figure 1.

The chromatographic peak shown in black looks Gaussian, but it is actually the result of at least three compounds that were only partially resolved. The spectrum at the apex of this peak is composed of ions from all three compounds, some of which are common to two or three of the overlapping analytes. AMDIS deconvolutes the chromatogram and pulls out "cleaned" spectra from the overlapping peaks. In most cases AMDIS is very successful at isolating a compound's spectrum from column bleed, other analytes, and coextracted interferences, even when interference abundances are far greater than the target analyte.

Using the deconvoluted full spectrum, AMDIS searches each peak against Agilent's Japanese Positive List Pesticide Library and reports a hit if the match quality exceeds a user-settable threshold. Since compounds are also required to have the

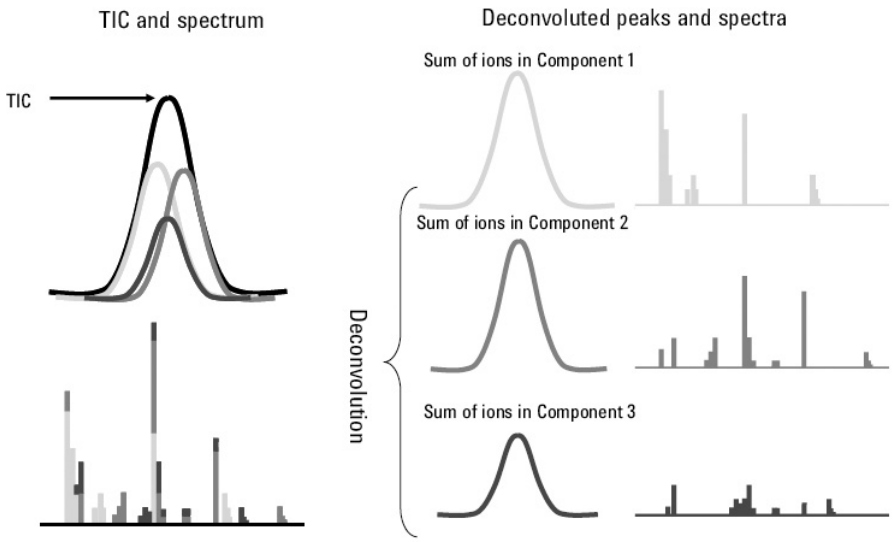

Figure 1. An illustration of the mass spectral deconvolution process.

correct retention time false positives are virtually eliminated. At the same time, false negatives are minimized because AMDIS deconvolution is able to generate library searchable spectra, even for traces of pesticides that are obscured by heavy matrix.

As a confirmation step, the deconvoluted spectra of all AMDIS hits are searched against the 163,000-compound NIST mass spectral library; for this step, there is no retention time requirement. More details about DRS can be found in earlier publications [11-17]. Experience in this laboratory and others [14, 15] has shown that DRS is the fastest, most comprehensive method for pesticide screening and that it produces the fewest false positives and false negatives.

Pesticides Included in the Japanese Positive List Pesticide Database

Pesticides included in the Japanese Positive List Pesticide Database were derived from three sources. First are the pesticides listed for analysis by GC/MS under the current MHLW regulations. Some additional pesticides were added because they were published in a recent paper written by analysts from the Kobe and Yokahama Quarantine Stations[18]. A third source of pesticides came from the Japanese Office of Food Safety's "Imported Foods Monitoring Plan for FY 2006." This plan says that imported foods and agricultural products must "conform with Schedule 6" of this document, which is a list of 447 pesticides [19]. Of the 447 pesticides, some are better analyzed by GC and others by LC. Some are amenable to either method, but the document does not specify the methods to be used. Agilent's Japanese Positive List Pesticide Database includes all of the compounds in this list that can realistically be analyzed by GC/MS.

The actual MRL values appear in two lists – one containing the finalized MRLs [4] and another with provisional MRLs [5]. On February 5, 2007, the Japanese MHLW published revised versions of both MRL lists. Sixty-seven new drugs and pesticides were added to the revised provisional list.

Of the pesticides in the original Japanese Positive List, 265 were to be analyzed by GC/MS. Many of the 59 newly added pesticides are also amenable to GC/MS analysis. The resultant Japanese Positive List Pesticide Database contains the mass spectra and locked retention times for 430 pesticides and one internal standard (phenanthrene-d_{10}).

Analyzing Samples

Figure 2 shows a chromatogram of a strawberry extract that was spiked with eight pesticides (500 ng/g each) and analyzed using the Japanese Positive List method. The first 18 minutes of the chromatogram are very "dirty," with many large peaks from endogenous compounds that were extracted along with the pesticides.

DRS was run on this sample but the GC/MS was not calibrated for the target compounds. Instead,

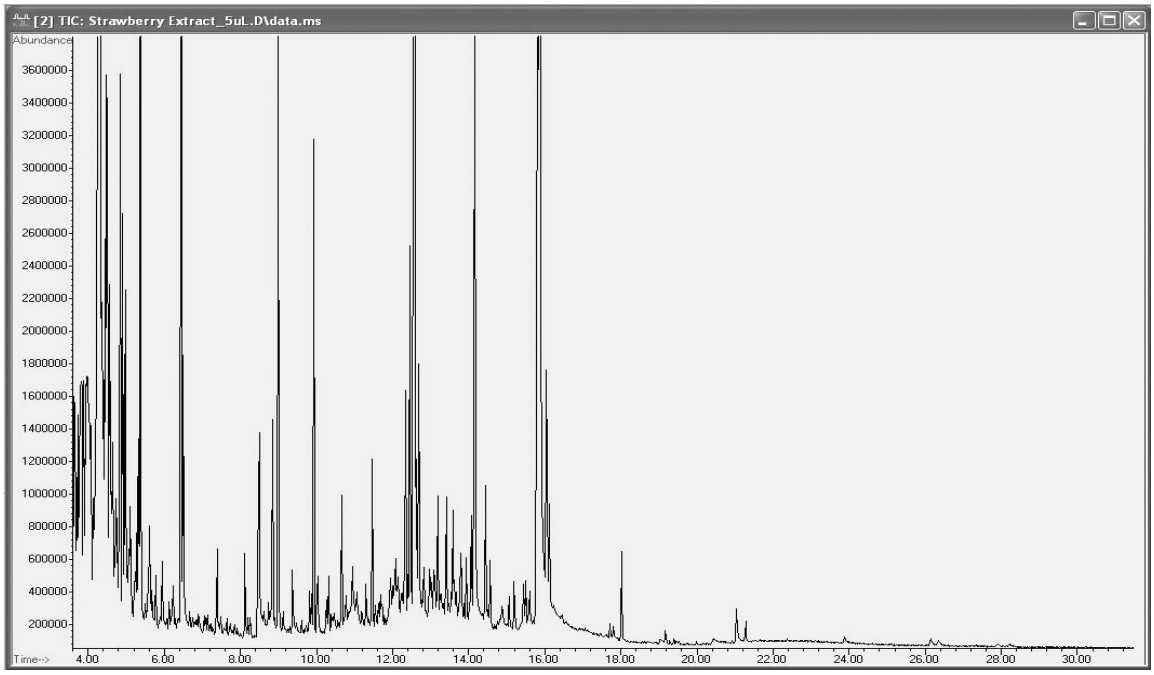

Figure 2. Chromatogram of a strawberry extract that was spiked with eight pesticides each at 500 ng/g. Seven of the eight pesticides are in the Japanese Positive List Pesticide Database and were easily identified by DRS.

an average response factor was used for all pesticides in the database. Figure 3 shows the report that was generated by DRS in about 90 seconds.

Temephos was spiked into the sample but was not found by DRS because this compound is not in the database. Pyridaben was identified by the Agilent ChemStation but was not confirmed by AMDIS. The ChemStation uses four ions for identification while AMDIS uses the whole deconvoluted spectrum. It is rare that the ChemStation identifies a target compound that AMDIS doesn't find. A quick review of the data showed that pyridaben was not present in the sample. As with methiocarb, it is much more common for AMDIS to find compounds that are not reported by the ChemStation. This example shows how DRS helps to eliminate both false positives and false negatives.

For each of the seven hits, DRS provided the retention time, CAS number, and name of the pesticide. Column four shows the amount of each hit as determined by the ChemStation software. Agilent supplies four methods for use with the Japanese Positive List Pesticide Database that can be used with different instrument configurations. Each of these methods comes with a quant database for all 430 target pesticides. The response factors provided with these methods were derived by taking the average response factor for about 25 representative pesticides. These response factors are not correct, but allow the analyst to estimate concentrations of the target compounds. The estimated values are usually within an order of magnitude of the actual concentrations. For accurate quantitative analysis, laboratories must do their own calibrations. Since it is unlikely that a laboratory would calibrate for all 430 pesticides, they can use these average values for compounds that are not calibrated. When a new compound is identified by DRS, it can be added to the calibration solutions.

Column five shows how well the deconvoluted spectrum matches the target pesticide library spectrum (100 = perfect match). Also under the AMDIS heading is the retention time difference between the library and observed values (column 6). Because RTL was used in the creation of the library and for the strawberry analysis, the observed values are extremely close to the library. Remarkably, only one compound, fenamiphos, deviated from its library retention time by more than one second.

The last step in the DRS analysis is to compare the

MSD Deconvolution Report

Sample Name: Strawberry extract

Data File: C:\msdchem\1\DATA\Strawberry_TID_2uL.D

Date/Time: 04:44 PM Thursday, Sep 6 2007

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent ChemStation	AMDIS		NIST	
R.T.	Cas #	Compound name	Amount (ng/µL)	Match	R.T. Diff sec.	Reverse match	Hit number
11.4914	298022	Phorate	0.99	95	–0.4	90	1
12.3647	13071799	Terbufos	1.4	97	–0.5	89	1
12.4698	333415	Diazinon	1.09	95	–0.3	80	1
12.5726	1517222	Phenanthrene-d_{10}		98	–0.6	84	1
12.7135	298044	Disulfoton	0.91	87	–0.3	84	1
14.0851	2032657	Methiocarb		85	0.5	81	1
14.4553	55389	Fenthion	2.57	99	–0.3	90	1
16.0453	22224926	Fenamiphos	3.32	96	2.4	88	1
20.630	96489713	Pyridaben	0.04				
12.575		Phenanthrene-d_{10}	5				

Figure 3. DRS report for the strawberry sample whose chromatogram is shown in Figure 2. Amounts shown in column 4 are only approximations, derived using an average response factor rather than individual pesticide calibrations.

deconvoluted spectrum for each hit to the entire NIST05 mass spectral library. If it finds the pesticide among the top 100 library hits, it prints the match value and the hit number in the last two columns. This step provides further verification of the compound's identity.

Figure 4 shows the chromatogram of a mixed fruit extract that was not spiked. DRS found two fungicides – diphenylamine and thiabendazole, along with one organophosphorus pesticide – azinphos-methyl (Figure 5). In the initial analysis, diphenylamine was not reported by the ChemStation even though it was an excellent match in AMDIS and to the NIST05 library. In this case, the diphenylamine peak was integrated manually using the Q-Edit feature of the ChemStation and DRS was rerun using the "existing quant" results. Figure 6 shows the new DRS report for the mixed fruit extract with quant results for diphenylamine. The reported values are only estimates because an average response factor was used for quantification.

Azinphos-methyl was identified in the mixed fruit

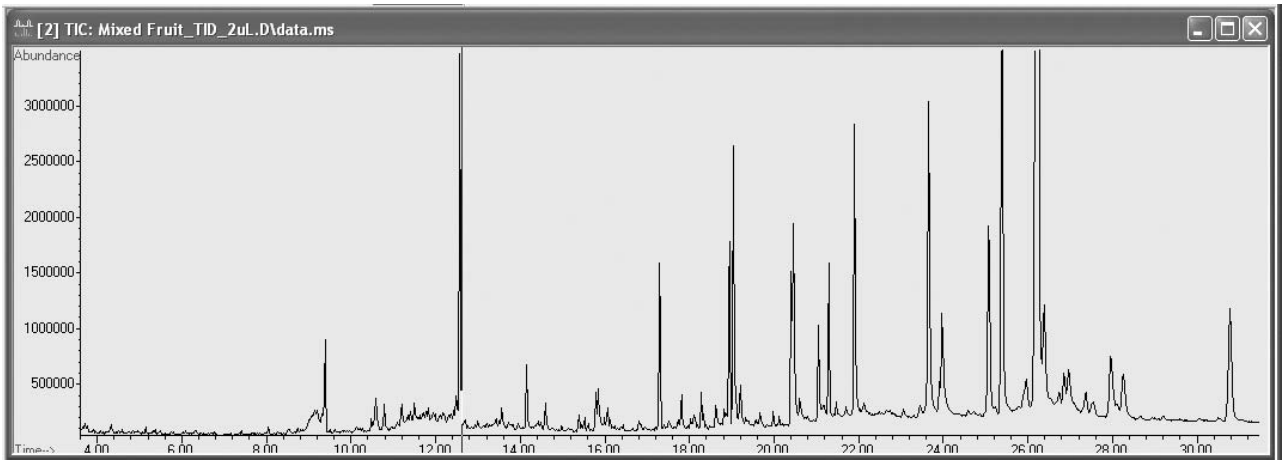

Figure 4. Chromatogram of an unspiked mixed fruit extract.

MSD Deconvolution Report

Sample Name: Mixed fruit

Data File: C:\MSData\Sept 04_07 Lehotay samples using TID & Japanese method\Mixed Fruit_TID_2uL.D

Date/Time: 04:01 PM Monday, Sep 10 2007

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent ChemStation	AMDIS		NIST	
R.T.	Cas #	Compound name	Amount (ng/µL)	Match	R.T. Diff sec.	Reverse match	Hit number
10.7853	122394	Diphenylamine		97	–0.1	91	1
12.5733	1517222	Phenanthrene-d_{10}		99	–0.6	84	2
15.3882	148798	Thiabendazole	0.66	99	–2.6	92	1
19.4523	86500	Azinphos-methyl	0.02	63	–0.6	76	2
12.575		Phenanthrene-d_{10}	5				

Figure 5. Initial DRS report for the mixed vegetable sample whose chromatogram is shown in Figure 4.

MSD Deconvolution Report

Sample Name: Mixed fruit

Data File: C:\MSData\Sept 04_07 Lehotay samples using TID & Japanese method\Mixed Fruit_TID_2uL.D

Date/Time: 04:11 PM Monday, Sep 10 2007

The NIST library was searched for the components that were found in the AMDIS target library.

R.T.	Cas #	Compound name	Agilent ChemStation Amount (ng/µL)	AMDIS Match	AMDIS R.T. Diff sec.	NIST Reverse match	NIST Hit number
10.783	122394	Diphenylamine	0.92	97	–0.1	91	1
12.5733	1517222	Phenanthrene-d_{10}		99	–0.6	84	2
15.3882	148798	Thiabendazole	0.66	99	–2.6	92	1
19.4523	86500	Azinphos-methyl	0.02	63	–0.6	76	2
12.575		Phenanthrene-d_{10}	5				

Figure 6. DRS report after using Q-Edit to integrate the diphenylamine peak. DRS was run a second time using the "existing quant" results.

extract even though it was buried by coeluting compounds. The benefits of deconvolution are apparent in Figure 7, which shows a screen capture from the AMDIS software. The arrow in Figure 7a shows where azinphos-methyl elutes. Figure 7b shows the spectrum at that point while Figure 7c juxtaposes the deconvoluted spectrum (white) with the library spectrum (black). Without deconvolution it would have been impossible to identify azinphos-methyl by library searching. Even knowing that azinphos-methyl was present, it was impossible to create a library-searchable spectrum by standard background subtraction techniques. After deconvolution, the spectrum is a reasonable match to the library.

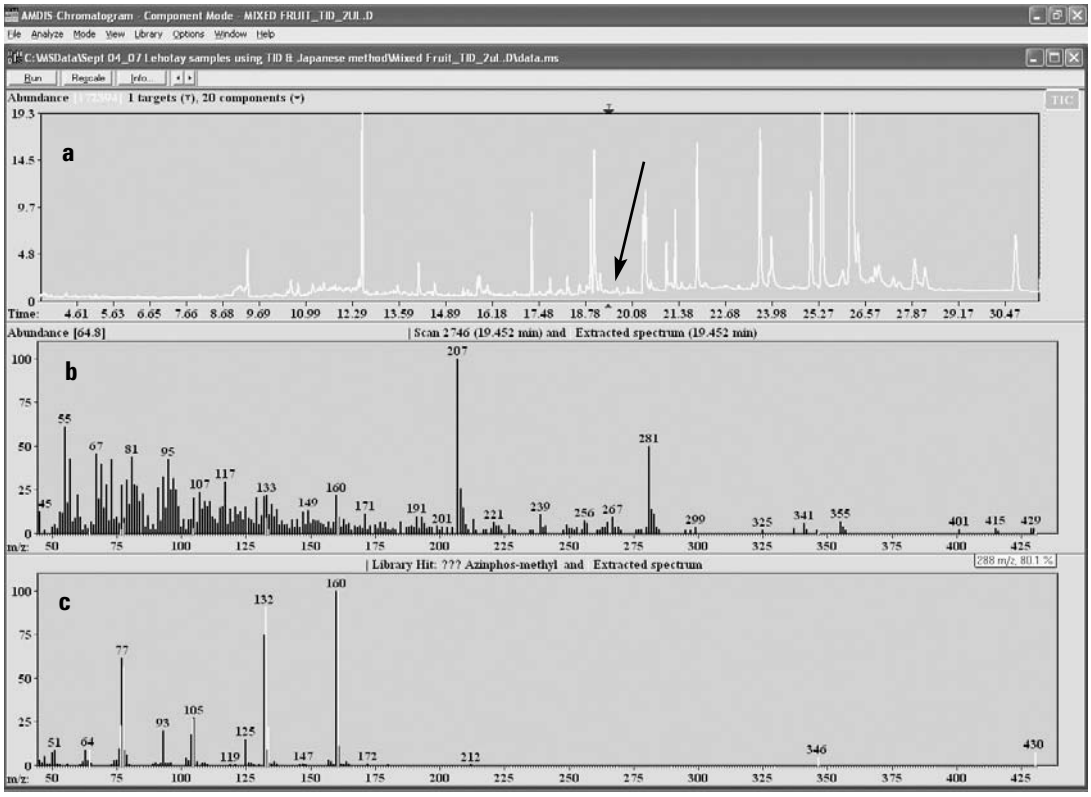

Figure 7. Screen capture from AMDIS showing: a) the total ion chromatogram of a mixed fruit extract, b) the spectrum where Azinphos-methyl elutes, and c) the deconvoluted spectrum (in white) juxtaposed to the library spectrum for Azinphos-methyl (black).

Several studies have shown that DRS is capable of identifying pesticides that are not found by conventional pesticide analysis [14, 15]. Most GC and GC/MS pesticide methods target a fixed number of compounds and generally do not identify compounds unless they are on the target list. Moreover, it can easily take a skilled analyst 30 minutes or more per sample to verify the analytical results. By contrast, Agilent's DRS method can screen for all of the GC-amenable pesticides of interest to the Japanese government (430 pesticides) in about two minutes.

Conclusions

Agilent Technologies has introduced a new retention time locked mass spectral database to address the needs of laboratories that must comply with the Japanese Positive List pesticide regulations. This database contains mass spectra in Agilent, NIST, and AMDIS formats along with locked retention times for 430 pesticides. The list of pesticides was derived from the most up-to-date publications of the Japanese Ministry of Health, Labour and Welfare and its agencies. The locked retention times were obtained using the GC/MS conditions recommended by the MHLW. Together with Agilent's Deconvolution Reporting Software, analysts can screen data files for all 430 pesticides in about two minutes per sample. The method is rapid, comprehensive, accurate, and automated so it depends less upon the skill of the individual analyst.

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Acknowledgements

The author would like to thank Drs. Steven Lehotay and Katerina Mastovska of the U.S. Department of Agriculture for providing the samples of spiked and unspiked extracts.

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Printed in the USA
September 21, 2007
5989-7436EN

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