A Rapid GC/MS Solution for the Japanese Positive List Pesticide Method

Application Brief

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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 it 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.

Food extracts for pesticide residue analysis are usually very dirty because one risks removing pesticides along with endogenous compounds if elaborate clean-up steps are used. So, the challenge is to detect traces of pesticides in samples that contain a lot of interferences. DRS helps the analyst "see through" the interferences and identify target compounds – pesticides in this case. The heart of DRS is software from the National Institute of Standards and Technology (NIST) called Automated Mass Spectral Deconvolution and Identification System (AMDIS). AMDIS profiles background noise, corrects for spectral skew, and interpolates between scans to find a more accurate retention time for every ion in every spectrum. It then looks for ions that rise and fall together and have the same retention time. These ions are grouped together into a "component" spectrum, which is searched against the Japanese Positive List Pesticide Library. In essence, AMDIS pulls clean, library-searchable spectra out of the background.

To illustrate the power of deconvolution, a green pepper extract was spiked with 229 pesticides, of which approximately 148 can be analyzed by GC/MS. The total ion chromatogram for this extract is shown in Figure 1. Figure 2 shows the undeconvoluted spectrum at 17.840 minutes (top) and the deconvoluted spectrum (middle). The deconvoluted spectrum is an excellent match to the fenhexamid library spectrum (bottom).

Highlights

- Deconvolution extracts clean, library-searchable spectra from coeluting peaks.
- Agilent's Japanese Positive List Pesticide Database and Deconvolution Reporting Software (DRS) enable screening of GC/MS data files for the 430 pesticides regulated by the Japanese government in a single run.
- Retention time locking eliminates almost all false positive hits.
- The fully automated process takes only 2 to 3 minutes per sample and is more reliable than conventional GC/MS methods.
- The DRS summary report quantifies calibrated compounds on the basis of their undeconvoluted and deconvoluted spectra.

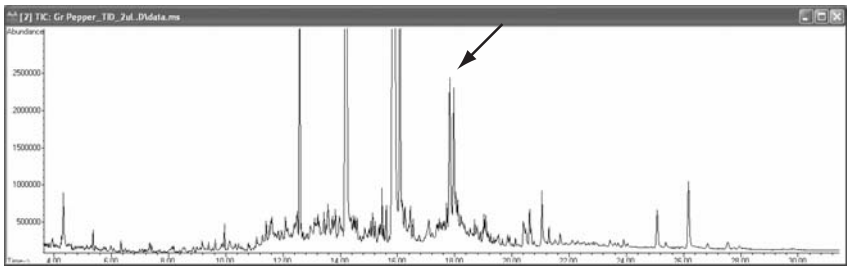

Figure 1. GC/MS total ion chromatogram (scan mode) of a green pepper extract spiked with 229 pesticides at 100 ppb each. The arrow shows where the pesticide fenhexamid elutes. The pesticide is buried under a much larger unresolved peak.

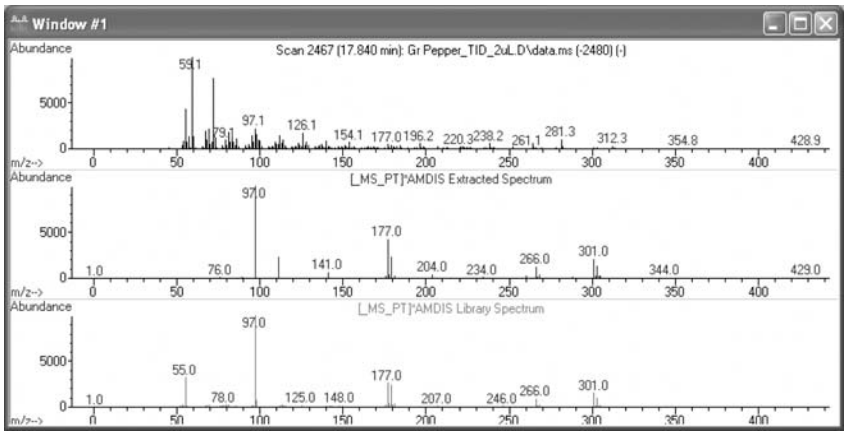

Figure 2. Top: Undeconvoluted spectrum taken at 17.840 minutes for the green pepper extract shown in Figure 1. Middle: Deconvoluted spectrum at 17.840 min. Bottom: Japanese Positive List Pesticide Library spectrum of fenhexamid.

Figure 3 shows part of a DRS report for the spiked green pepper extract. Large portions of the table were removed since it is shown simply to illustrate the table's format and the information it provides.

The first three columns give the retention time, CAS number, and name for each target analyte found by the DRS software. The next two columns show the calculated amount determined by the Agilent GC/MS ChemStation. The first value (column 4) is determined using the undeconvoluted target ion and represents the typical result one gets from a standard GC/MS calibration method. The amount under the "AMDIS" heading uses the deconvoluted target ion for its calculation.

Note that the quant results shown in Figure 3 are only estimates based on an average response factor that was used for all pesticides in the calibration table. Compounds were not calibrated individually. Many laboratories prepare a multilevel calibration for a list of target pesticides and use average calibration factors for the remaining compounds.

MSD Deconvolution Report

Sample name: Gr pepper
Data file: C:\MSData\Sept 04_07 Lehotay samples using TID & Japanese method\Gr Pepper_TID_2uL.D
Date/time: 1:59:58 PM Monday, July 14, 2008

Adjacent peak subtraction = 2
Resolution = High
Sensitivity = Very high
Shape requirements = Low

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

			Amount (ng/uL)		AMDIS		NIST	
R.T.	**CAS #**	**Compound name**	**Chem-Station**	**AMDIS**	**Match**	**R.T. diff sec.**	**Reverse match**	**Hit num.**
6.4699	10265926	Methamidophos	0.19	0.17	82	2.4	88	1
6.6154	62737	Dichlorvos	0.13	0.10	84	1.2	86	1
7.2062	1563388	Carbofuan-7-phenol	0.07	0.05	88	1.0	80	1
15.397	32809168	Procymidone	0.28	0.22	87	–0.5	83	1
15.407	148798	Thiabendazole	0.46	0.37	67	–1.5	66	1
18.217	51036	Piperonyl butoxide	1.02	0.15	97	–0.6	90	1
18.354	106325080	Epoxiconazole	0.35	0.16	86	–0.3	–	–
18.355	52669928	1-Butanone, 4-[4-(4-chlorophenyl)-1,2,3,6-tetrahydro-1-pyridyl]-1-(4-fluorophenyl)-	–	–	–	–	59	1
18.532	36734197	Iprodione	0.04	0.07	44	–0.5	58	11
18.539	119120	Pyridaphenthion	0.25	0.20	82	–0.2	66	1
18.573	135410207	Acetamiprid	0.05	0.03	46	–0.1	52	1
18.696	82657043	Bifenthrin	1.39	1.06	96	–0.7	90	1
20.674	96489713	Pyridaben	1.04	0.84	92	–0.7	90	1
20.682	67747095	Prochloraz	0.10	0.04	69	–0.8	60	1
24.042	110488705	Dimethomorph-(E)	0.16	0.15	88	–1.4	86	1
12.578		Phenanthrene-d10	10.00	–	–	–	–	–

Figure 3. Partial DRS report for a green pepper extract spiked with 229 pesticides at 100 ppb each. Some of these are not GC-amenable. Of the 229 spiked pesticides, 156 were found by this GC/MS method. Only a portion of the report is shown. The quantitative results shown in columns 4 and 5 are only approximations based on an average response factor. No individual compound calibrations were performed.

The column labeled AMDIS/Match shows the quality of the spectral match between the deconvoluted spectrum and the Japanese Positive List Pesticide Library spectrum. The reporting threshold can be set by the user. Matches below the threshold are not reported.

The Japanese Positive List Pesticide Database contains retention times for each pesticide using the published Japanese positive list method. With retention time locking (RTL), users can easily reproduce the database retention times. AMDIS can be configured to reject a "hit" if the compound's retention time falls outside of a user-settable window (typically ±10 sec). This feature greatly reduces false positive hits.

The next column in Figure 3, labeled AMDIS/R.T. Diff sec, shows the difference between the retention time of the analyte in the chromatogram and its value stored in the Japanese Positive List Pesticide Library. Of the 156 pesticides identified by DRS, fewer than 10 had retention times more than 2 seconds away from their database values. In extremely dirty samples, peaks may shift a bit but the retention time window can be widened to accommodate them. Even then, the retention time requirement eliminates almost all false positives.

As a general rule, matches greater than 60 are very reliable, especially when there is also a good match to the NIST library. For the DRS report shown in Figure 3, the minimum match value was set to 40. Several pesticides were reported with AMDIS match factors between 40 and 60. For these, it is good to review the results in Qedit, which now (using DRS version A.04 or greater) includes AMDIS results combined with the ChemStation results.

As a confirmation step DRS takes the deconvoluted spectrum for each "hit' and searches it against the entire NIST mass spectral library using the NIST library searching algorithm. If the hit is found in the NIST library, its match value and rank are reported. A "hit number" of 1 means that the compound identified by AMDIS was also the best match in the NIST library.

Much more information about this method may be found in an earlier Agilent Application [1]. The benefits of DRS Version A.04 are discussed in a recent Agilent *eSeparation Times* article [2].

Summary

- This Applications Brief discusses a method to screen for the GC-amenable pesticides that are being regulated by the Japanese Ministry of Health, Labour and Welfare (MHLW) under the Positive List System.
- The new Japanese Positive List Pesticide Database contains mass spectra for 430 pesticides and their locked retention times.
- Deconvolution is used to extract clean, library-searchable spectra from co-eluting peaks.
- Deconvolution Reporting Software (DRS) compares all of the deconvoluted spectra to the Japanese Positive List Pesticide Database to find any spectral matches. Hits must also fall within narrow retention time windows, made possible by the use of retention time locking.
- Screening takes 2 to 3 minutes per data file and is more reliable than conventional GC/MS methods.
- DRS produces a summary report showing compounds identified by the ChemStation, AMDIS, and NIST library searching. Calibrated compounds are quantified on the basis of their undeconvoluted and deconvoluted spectra.
- This method screens for all 430 pesticides of interest to the MHLW in a single run.
- Reference 1 contains a complete description of the method.

References

1. Philip L. Wylie, "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," Agilent Technologies publication 5989-7436EN, www.agilent.com/chem.
2. Mike Szelewski, "I'm interested in quantitation using deconvolved areas. Are the advantages of DRS Version A.04 great enough to justify learning this new software?" *eSeparation Times*, 21(2), www.agilent.com/chem.

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