application

Agilent Technologies
Innovating the HP Way

GC/MS

RTL-MSD Pesticide Method and Database

Efficient Screening for Pesticides and Endocrine Disrupters Using the 6890/ 5973 GC/MSD System

Introduction

Retention-time locking (RTL) is a new technique by means of which the GC can precisely and accurately reproduce compound retention times from one chromatographic analysis to another on the same gas chromatograph/mass spectrometer detector (GC/MSD) or on any similar GC/MSD system in any laboratory. RTL allows the chromatographer to develop and export methods that can be universally reproduced on similar systems. RTL is easy on the Agilent Technologies 6890 GC/ 5973 MSD system, and to complete the system, MSD Productivity ChemStation software provides RTL functionality as well as instrument control, data analysis, reporting, and mass spectral library matching.

Using RTL, a GC/MS method has been developed that allows any analyst to analyze for 567 of the most common pesticides and endocrine disrupters known to be in use worldwide. By using the RTL functionality with the 6890/ 5973 GC/MSD system, it has been possible to develop a new database containing both mass spectra and an index of compound retention times. When used with the compound screening tools included with the MSD Productivity ChemStation software, data files can be easily, accurately, and quickly screened for the presence of any of the 567 compounds listed in the database. In addition, software features make it possible to detect compounds that might ordinarily be masked by others.

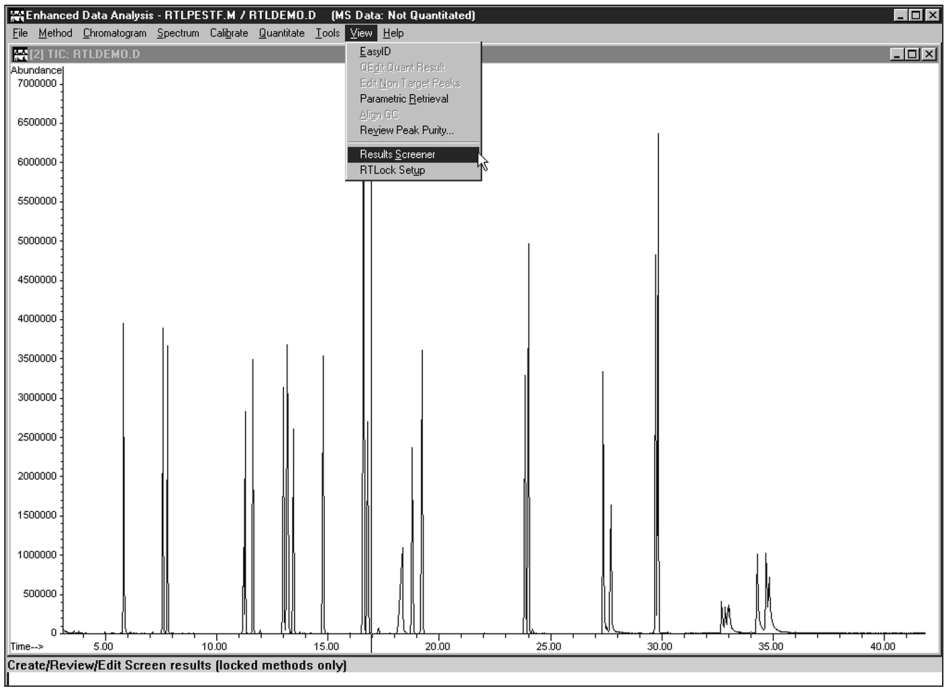

Figure 1. Selecting the Results Screener option from the View menu in the Enhanced mode of data analysis.

The GC/MSD RTL Method [RTLPEST.M]

The GC/MSD RTL method is a universal pesticide and endrocine-disrupter method that accommodates many different injection schemes—for example, cold on-column, pulsed split/splitless, large-volume injection with PTV and more. However, because the method is based on retention times, the column used must be an HP-5 MS column (30-m, 0.25-mm i.d., 0.250 um thickness—available as 190913-433). The HP-5MS columns are highly uniform in terms of specifications and performance. The 6890 GC oven parameters were chosen to provide the best resolution for the wide variety of chemicals that are potentially present in complex samples. The total pesticide run time for the HP-5 MS column is short, only 41.87 min. The pesticide method is locked on the compound chlorpyrifos-methyl at a retention time of 16.596 min using the automated RTL Setup feature included with the MSD Productivity ChemStation software. For more information on how to acquire RTL data, see Publication 5968-3433E.

Using The RTL MS Pesticide Library and Screener Software

In addition to the 6890/5973 GC/MSD system, the RTL-MSD Pesticide Library and RTL-GC/MSD acquisition screening method are required. The RTL-MSD Pesticide Library (RTLPEST.L) and acquisition method (RTLPEST.M) are ordered and installed separately (product number G1049A). Installation instructions for the RTL-MSD Pesticide Library can be found on the MS Library CD ROM. The pesticide acquisition method is loaded by using the MSD Productivity ChemStation software. For additional information regarding the use of the MSD Productivity ChemStation software, see Publication 5968-3433E and On-Line Help.

After the method is locked on chlorpyrifos-methyl and a sample acquired by using the locked RTL pesticide method, the RTL Results Screener option is selected from the View menu in the Enhanced mode of data analysis to invoke the screening program for the loaded data file. See Figure 1.

How Does It Work?

The Results Screener software automatically scans through the sample data file, comparing the acquired MS data with those of the 567 compounds in the RTL pesticide library. The data are compared with the target ion fragment information in the library, as well as with the expected retention time of each library compound.

The upper left window in Figure 2 displays the extracted ion chromatogram (EIC) for the sample based on the ions for the indicated compound. Compound matches (“hits”) that are confirmed with high confidence are indicated by an “X” in the Quick Screener compound results list (Figure 2a) Possible and questionable hits are indicated by a question mark (“?”) and suggest that further review of the data is appropriate. Using the Quick Screener tool, the analyst can automatically scroll through every compound in the library by pressing Start in the Quick Screener panel or can manually scroll to individual compounds of interest for further review.

Enabling the Display Reference Spectra function allows the analyst to simultaneously view the acquired mass spectrum versus the library's reference spectrum. See Figure 2b. The window located at the lower right of Figure 2 contains the compound name, retention time, deviation from the expected locked retention time, and the compound's target, actual, and expected ion ratios. See Figure 2c.

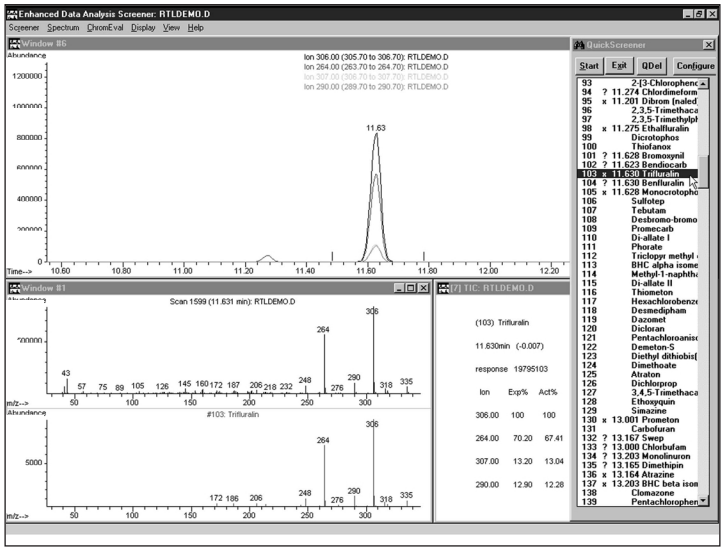

Figure 2. Results for GC/MS data acquired by the locked method, RTLPEST.M, as presented by the Results Screener view. The top window includes the extracted ion chromatogram for an unknown compound tentatively identified as trifluralin.

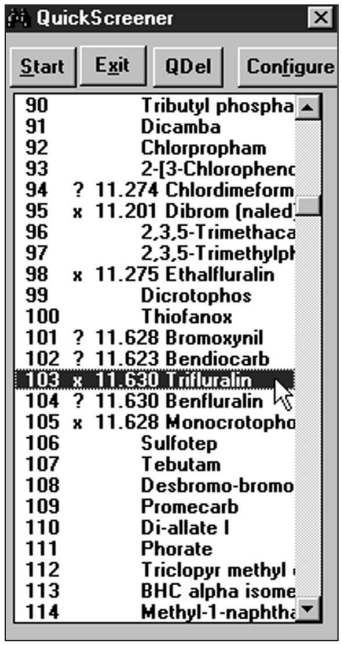

Figure 2a. Expanded view of right-hand window in Figure 2. Compound confirmation is denoted by an "X," and uncertain identification is denoted by a question mark ("?"). The "?" notifies the operator that further evaluation of a compound may be in order.

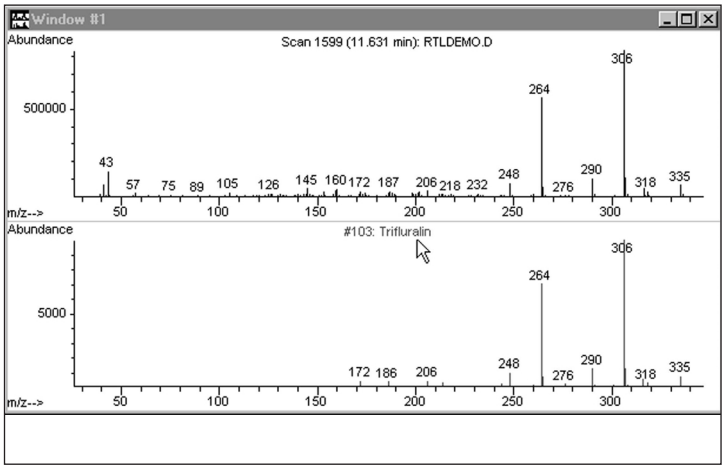

Figure 2b. Expanded view of lower left-hand window in Figure 2. Example of actual (upper) and library (lower) spectral comparison.

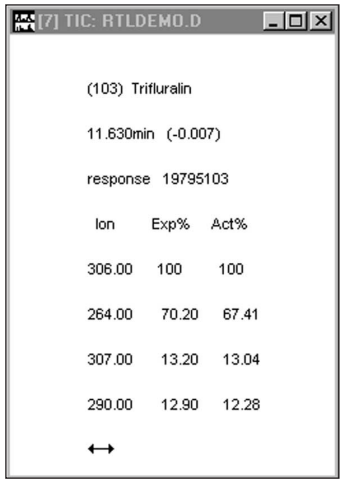

Figure 2c. Expanded view of lower right-hand window in Figure 2. Compound information provided includes compound name, the retention time and deviation from the expected locked retention time, and the compound's target ions and their actual and expected ratios.

As mentioned previously, positive hits are denoted by an "X" in the Quick Screener list, and possible or questionable identifications are denoted by a question mark ("?"). For example, although benfluralin co-elutes with trifluralin and contains common ions, the Screener software correctly indicates the failure of the qualifying ions. Upon closer inspection of the Quick Screener list shown in Figure 3a, the spectral comparison shown in Figure 3b, and qualifier ion table shown in Figure 3c, one can easily discern that the qualifier ions are not present. This information provides the user with high confidence that there is no detectable benfluralin in the sample. See Figure 3.

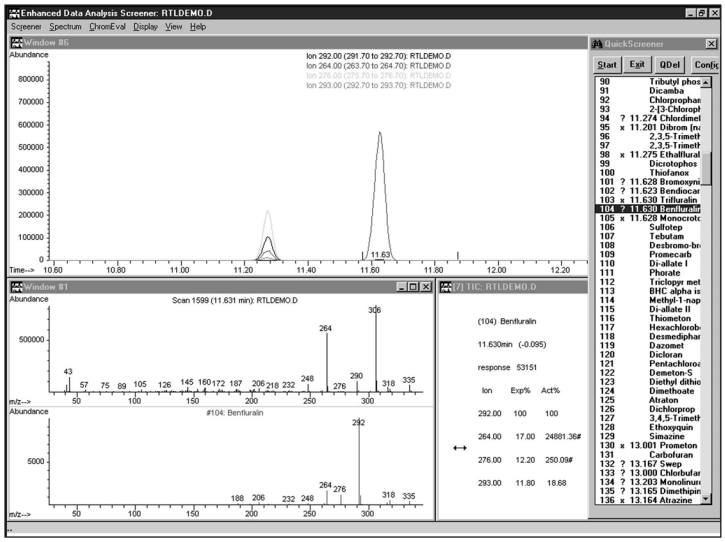

Figure 3a. The Quick Screener list informs the user of a questionable ("?") hit for benfluralin.

Figure 3. Evaluation of the possible presence of benfluralin.

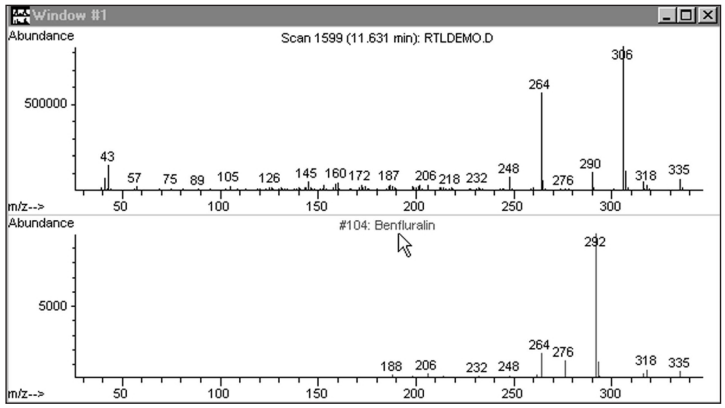

Figure 3b. A spectral comparison confirms the absence of the m/z = 292 qualifier ion in the actual sample spectrum. In addition, the m/z = 264 ion ratios are inconsistent.

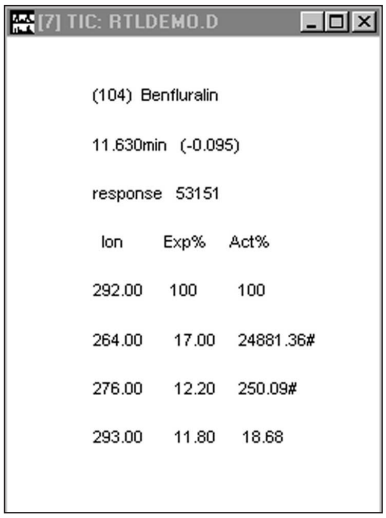

Figure 3c. The qualifying ion list clearly identifies the two ions that have failed to meet the expected qualifier ion ratios as indicated by the "#" sign.

Co-Eluting Compounds

Another utility of the Results Screener is discerning compounds that may be obscured by co-eluting chromatographic peaks. In Figure 4a, the Screener software suggests a hit for the compound chlorpyrifos-methyl, but there is an unexplained ion fragment at m/z = 263 and a skewing of the ion ratios for the m/z = 125 ion. By selecting a compound with a close retention time and similar ions as indicated by the "?" in the Quick Screener list, the m/z = 263 ion fragment and skewing of the m/z = 125 ion can be explained as co-elution with methyl parathion. See Figure 4b.

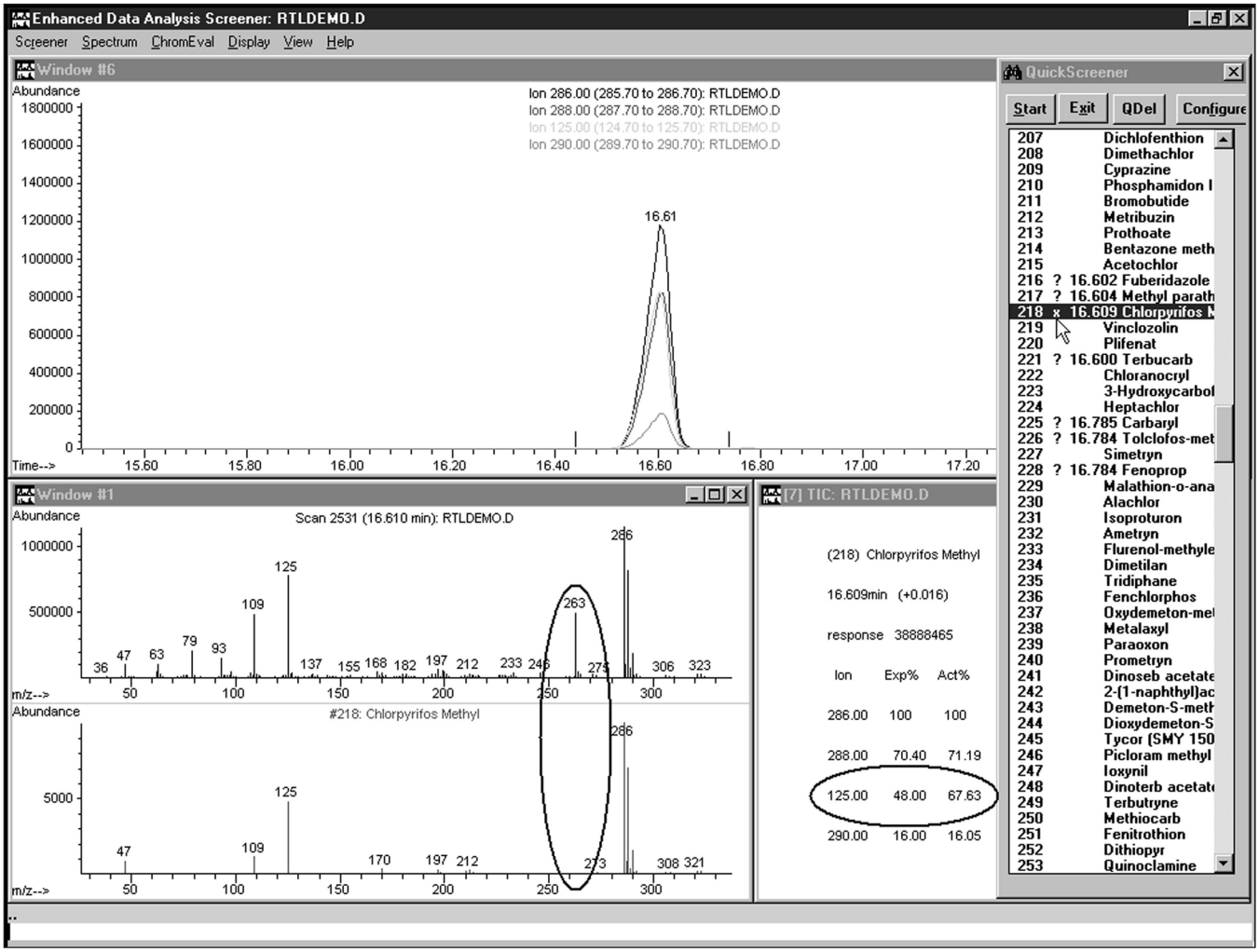

Figure 4a. Detection of possible compound co-elution is assisted by information presented in the Result Screener software. The Result Screener indicates a hit for the compound chlorpyrifos-methyl. However, closer examination of the spectral comparison and qualifier ion ratios indicate the possible presence of a co-eluting compound.

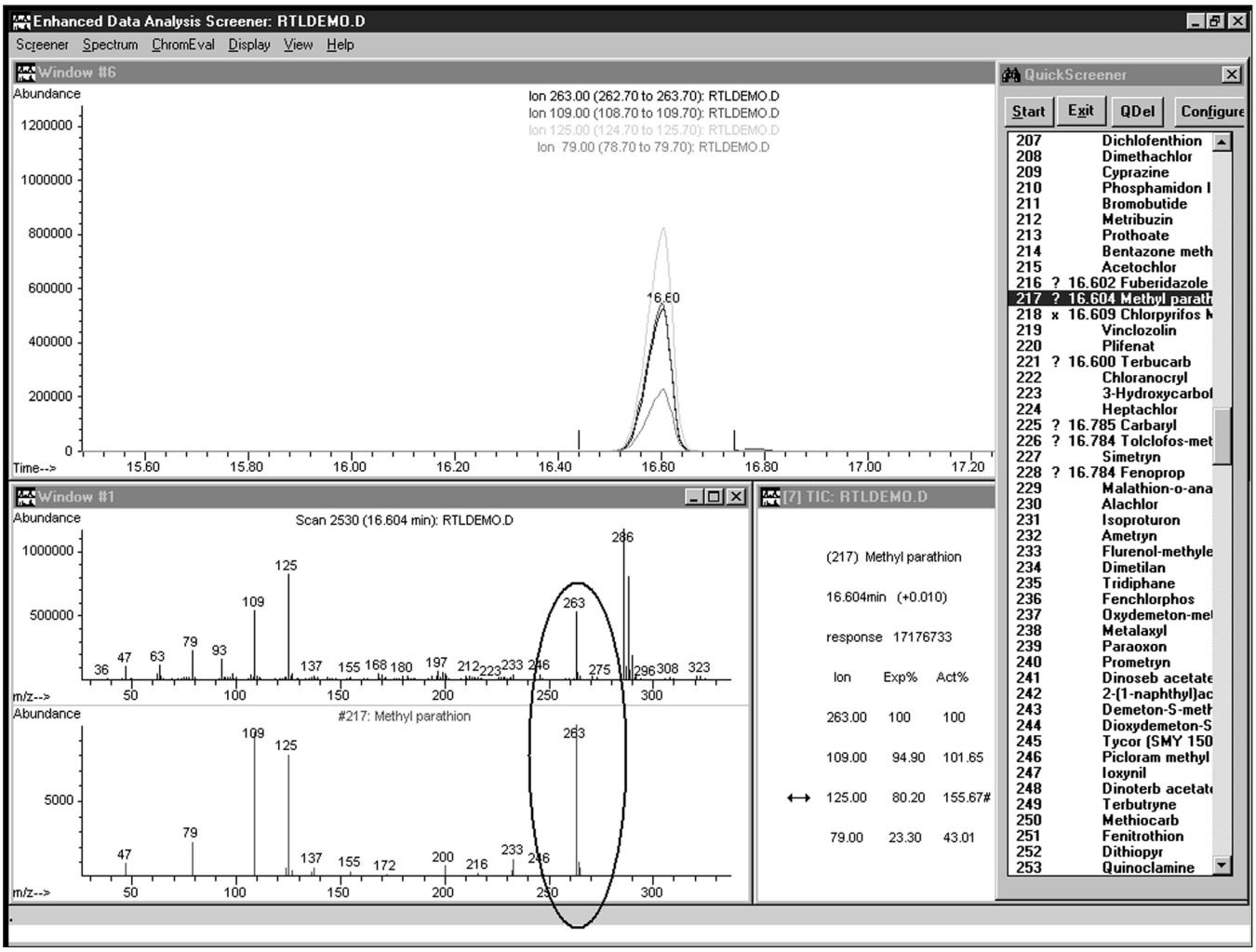

Figure 4b. Selecting the compound with the closest retention time in the Results Screener window, the spectral match and qualifying ion ratios identify the co-eluting peak to be methyl parathion.

Identifying Isomers

Many pesticide formulations consist of isomer mixtures. The classic example is the hexachlorocyclohexanes (BHCs). Because these are isomers, they have identical mass spectra and can only be distinguished by retention times. See Figures 5a and 5b.

The Screening software also automatically creates a report of possible and confirmed hits. See Figure 6. The report presents the experimental retention time, deviation from the expected locked retention time, target ions, response, and qualifier ions that fall out of range.

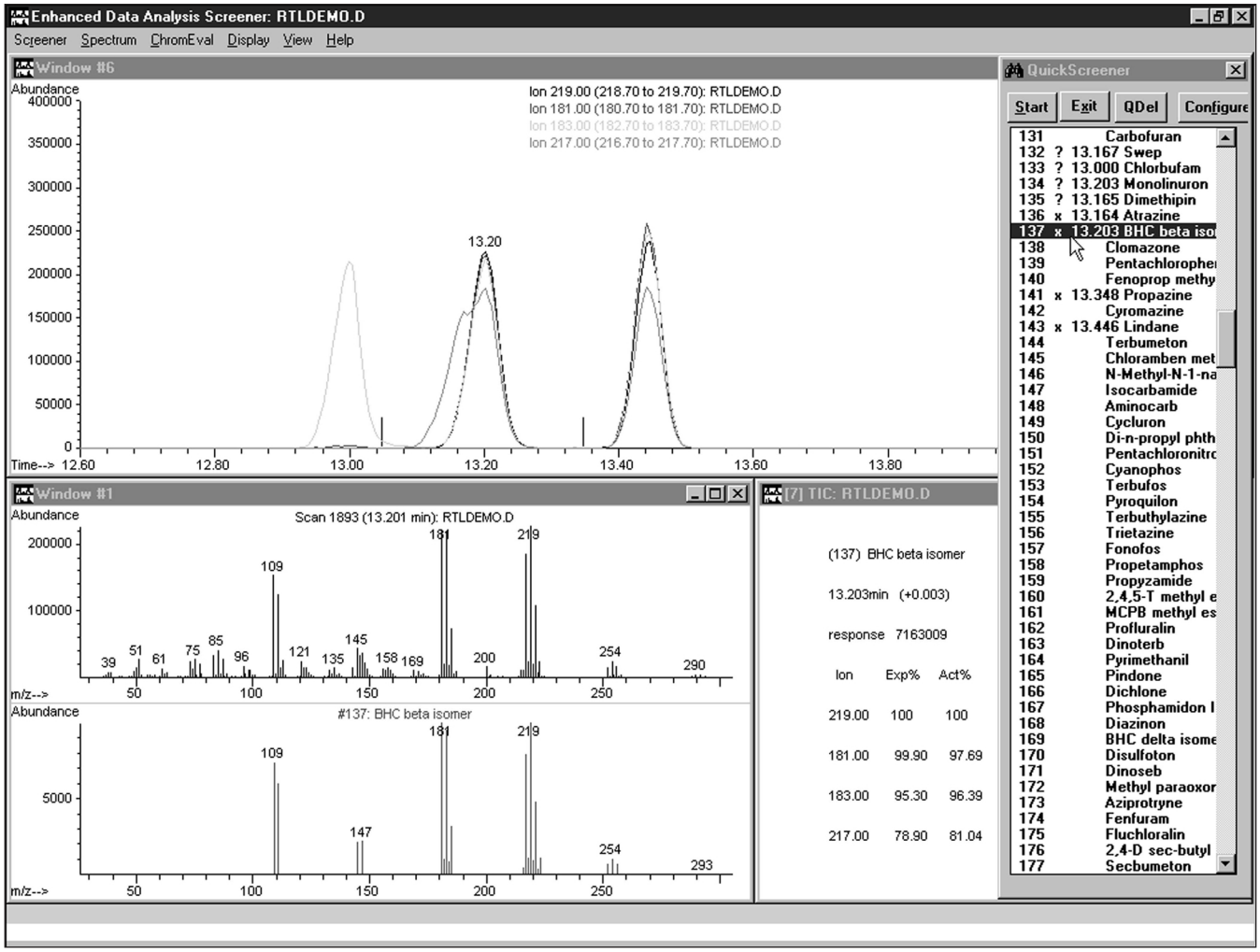

Figure 5a (above) and 5b (next page). The Screener software correctly identifies the two isomers using their respective retention times: the BHC beta isomer at 13.203 min and lindane (gamma BHC isomer) at 13.446 min.

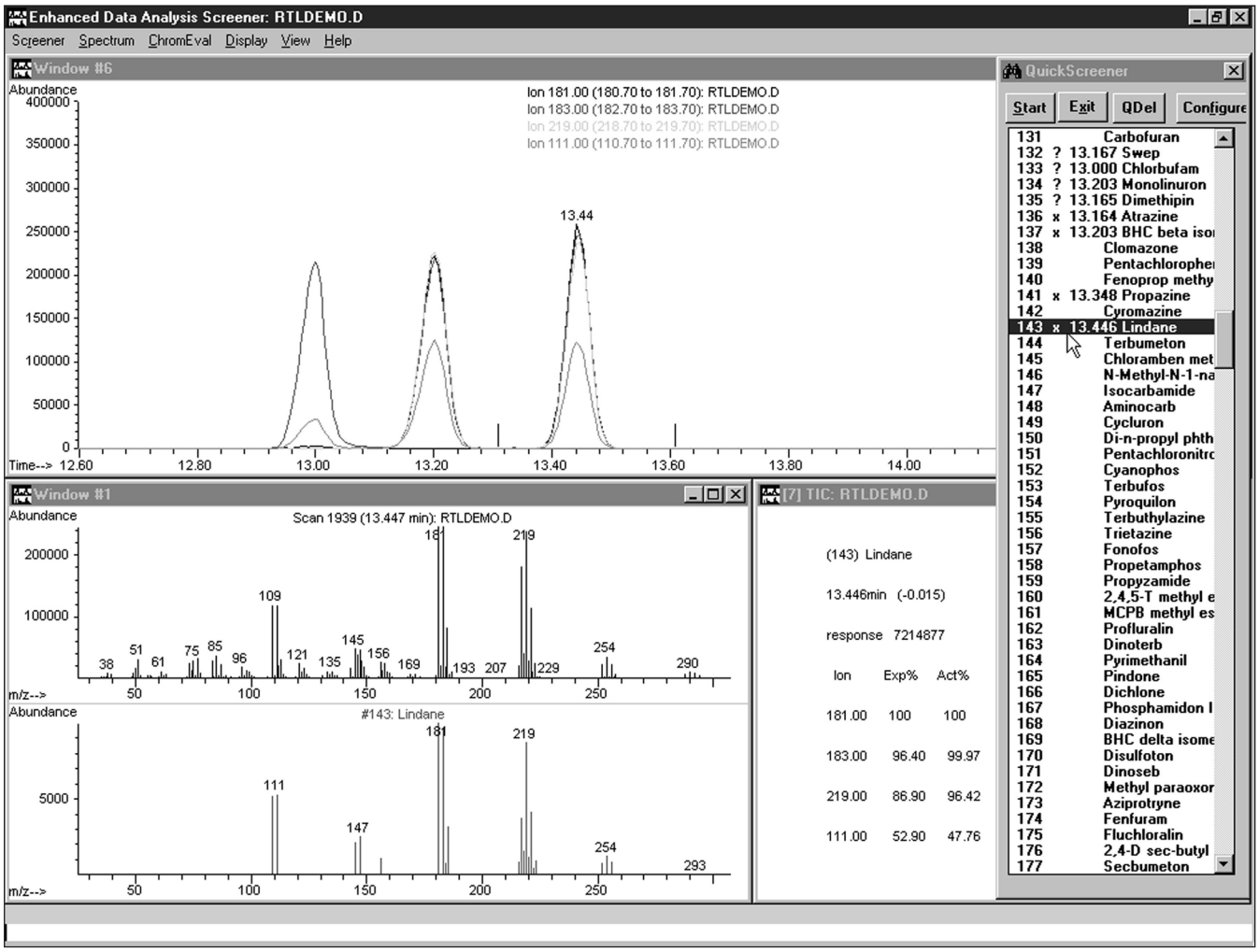

Figure 5b. See caption under Figure 5a on previous page for description.

MultiVu - [C:\HPCHEM\MSDEMO\RTLDEMO.D\SCRNTEMP.TXT]

File Edit Search Window

Screen Report

Data File : C:\HPCHEM\MSDEMO\RTLDEMO.D Vial: 4
Acq On : 5 May 1997 10:14 Operator: jig
Sample : TEST MIX1A Inst : GC/MS Ins
Misc : 26 pests, 10 ng/ul, 17.2 psi const p Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: events.e

Screen File: screen\RTLPEST.RES

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
21	Dichlorvos	x	5.831	-0.017	109	28872469		0.88
35	Mevinphos	x	7.595	-0.013	127	28860822		1.00
36	Butylate	?	7.607	-0.023	57	27318	146,156,174	0.00
37	3,4-Dichloroaniline	?	7.664	+0.122	161	-47520	163	0.09
40	Vernolate	x	7.806	-0.021	128	18049988		0.48
94	Chlordimeform	?	11.201	+0.073	196	111377	181,117,152	0.00
95	Dibrom (naled)	x	11.221	-0.020	109	9078031		0.94
98	Ethalfluralin	x	11.283	-0.008	276	4696405		0.84
101	Bromoxynil	?	11.550	+0.078	277	42935	279,88	0.00
102	Bendiocarb	?	11.540	+0.083	151	157380	126,166,57	0.00
103	Trifluralin	x	11.637	-0.007	306	19795103		0.96
104	Benfluralin	?	11.725	-0.095	292	53151	264,276	0.10
105	Monocrotophos	x	11.737	-0.109	127	196902		0.00
130	Prometon	x	12.990	+0.011	210	13930019		0.95
132	Swep	?	13.052	+0.115	187	19798	189,219	0.00
133	Chlorbufam	?	13.063	-0.063	153	1912228	127,53	0.08
134	Monolinuron	?	13.098	+0.105	61	165473	153,127	0.02
135	Dimethipin	?	13.098	+0.067	54	103136	39,53	0.06
136	Atrazine	x	13.159	+0.005	200	15570363		0.91
137	BHC beta isomer	x	13.200	+0.003	219	7163009		0.83
141	Propazine	x	13.363	-0.015	214	108048		0.75
143	Lindane	x	13.461	-0.015	181	7214877		0.91
179	Chlorothalonil	x	14.784	-0.000	266	26682153		0.66
216	Fuberidazole	?	16.549	+0.053	155	686802	156	0.00
217	Methyl parathion	?	16.594	+0.010	263	17176733	125	0.41
218	Chlorpyrifos Methyl	x	16.593	+0.016	286	38888465		0.52
221	Terbucarb	?	16.686	-0.086	205	160687	220	0.00
225	Carbaryl	?	16.806	-0.021	144	54379	115,116,145	0.00
226	Tolclofos-methyl	?	16.808	-0.024	265	446601	267,266	0.00
228	Fenoprop	?	16.801	-0.017	196	602537	198,97	0.03
260	Bromacil	x	18.350	-0.003	205	15483082		0.95
263	Di-n-butylphthalate	?	18.415	-0.015	149	551586	205	0.00
269	Oxamyl	?	18.728	+0.062	44	50069	98,72,69	0.01
270	Malathion	x	18.800	-0.011	173	12954421		0.41
273	Fenthion	?	19.120	+0.106	278	377014	125,109,169	0.00
276	4,4'-Dichlorobenzophenone	?	19.201	+0.021	139	47474	111,141,250	0.00
277	Tetrapropyl thiodiphospha	?	19.212	+0.015	211	475326	210,253	0.01
278	Chlorpyrifos	x	19.234	-0.009	197	10194282		0.50
280	Parathion	?	19.275	-0.058	291	9017	109,97,139	0.00

Figure 6. Example of pesticide screener report.

Conclusions

The highly accurate and reproducible pressure and temperature control of the 6890 GC made it possible to create a mass spectral library and a retention time-locked database of 567 chemicals of concern. The MSD Productivity ChemStation software provides a Screener tool that automatically presents concise and complete information for identifying and confirming the presence of target compounds utilizing both mass spectral and retention-time information. When used together, analyst can rapidly survey for the presence of any of the 567 compounds with both mass spectral and chromatographic confidence in compound identity.

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