RESIDUES AND TRACE ELEMENTS

Quantitative Identification of Pesticides as Target Compounds and Unknowns by Spectral Deconvolution of Gas Chromatographic/Mass Spectrometric Data

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The results of gas chromatography/mass spectrometry (MS), with Ion Signature Technology, Inc. (North Smithfield, RI) quantitative deconvolution software, are discussed for pesticides identified both as target compounds by using retention and MS data and as unknowns by using only mass spectra. Target compound analysis of 32 pesticides, surrogates, and an internal standard added to lemon oil over a wide concentration range produced precision and accuracy that are well within the acceptable criteria of 25 and 50% for complex samples. When 112 pesticides were added to orange oil and searched as unknowns, 110 of the 112 compounds were correctly identified, with an average pesticide recovery of 101 ± 19%. The injection volume of the orange oil fortified with pesticides was selected so that 4 ng per compound was injected on column. No false negatives were found, because ion signals for the 2 unidentified pesticides were not acquired by the instrument in either the standard mixture or the oil. No false positives were detected, although >750 widely different compounds were included in the library search.

For nearly 100 years, scientists have relied on mathematics and statistics to process signals and simplify data produced by analytical instruments. Although sophisticated techniques are routine, the trend toward faster, more sensitive analyses has propelled chemometric advances toward distinguishing a compound's mass spectrum from instrument or chemical noise. These improvements are due to a confluence of events, which have led to significant increases in the number of analytes examined, the number of samples analyzed, and the quality of data obtained, namely: (*1*) federal regulations; (*2*) forensic investigations; (*3*) small molecule drug discovery, development, manufacture, and compliance; and (*4*) protection of brand equity (e.g., product profiling and quality control; QC). To meet these needs, instrument manufacturers strive to provide higher sample throughput rates and lower method detection limits. Conspicuously missing are parallel improvements in software aimed at providing fewer false positives/negatives and easier ways to QC complex data files.

All gas chromatography/mass spectrometry (GC/MS) companies integrate their data analysis software with databases that contain libraries of mass spectral information. The National Institute of Standards and Technology (NIST), Wiley, and others provide excellent libraries, but experience shows that current search-engine software offers little advantage when highly complex, concentrated mixtures are analyzed. Toward this end, 2 MS companies promote deconvolution as a central technology in their data analysis software. Deconvolution is the mathematical separation of a target compound's mass spectrum from chemical and instrumental noise (1). Automated Mass Spectral Deconvolution and Identification System (AMDIS), developed by NIST and incorporated by Agilent Technologies (Santa Clara, CA) into its data analysis software, extracts mass spectra and fits a least-squares regression model to the compound's deconvoluted ion current chromatogram. The process involves 4 sequential steps: (*1*) noise analysis; (*2*) component perception; (*3*) spectrum deconvolution; and (*4*) compound identification (2–4). It appears that AMDIS does not have sufficient sensitivity to unambiguously identify low-concentration pesticides in high-concentration matrixes unless at least 3 clean scans exist in the total ion current (TIC) peak, with the scan rate optimized to increase data density while ion skewing is minimized (5–7). For quantitative results, the current Agilent Technologies software uses its own software to independently extract the peak area and

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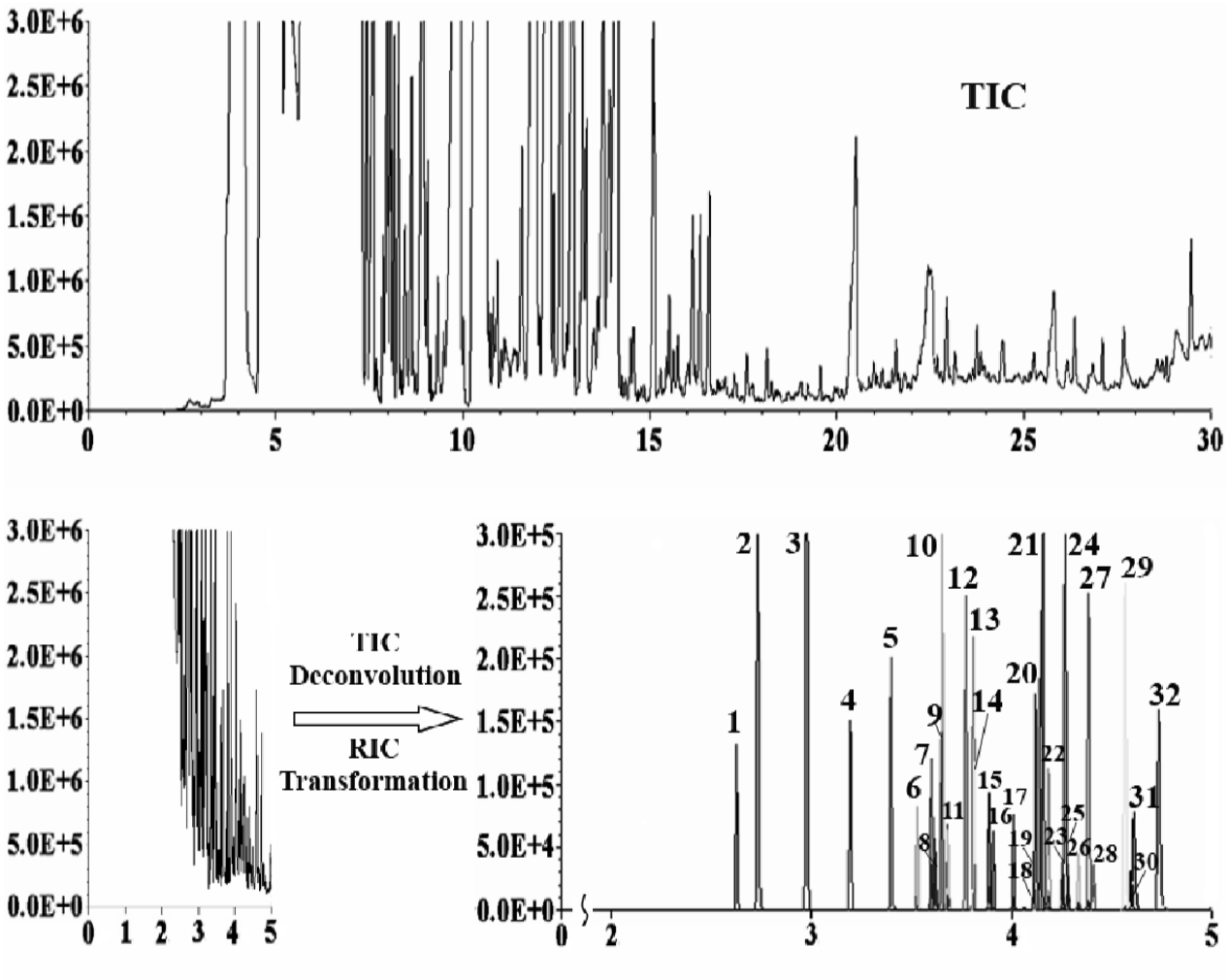

Figure 1. Total ion current (TIC) chromatograms for lemon oil and fortified lemon oil under slow and fast GC/MS conditions. The reconstructed ion current chromatogram is the transformed TIC signal for the 32 target compounds after deconvolution; *see* Table 1 for peak identities.

calculate the concentration. Agilent Technologies expects to introduce a new software product that will integrate the AMDIS extracted peak for quantitation. In contrast, LECO's (St. Joseph, MI) data analysis software is based on finding at least 1 unique target compound mass spectrum from peak scans in which chemical noise is minimal (8–10). Once the mass spectrum is found, a system of simultaneous equations is used to deconvolute the remaining peak scans. This process is more akin to chromatographic deconvolution than to "true" spectral deconvolution, and it requires the high-speed signal transmission of time-of-flight (TOF) instruments filters to provide the necessary data density (11). AMDIS, on the other hand, is used successfully with magnetic, quadrupole, and TOF filters. When combined with NIST's MS search library, deconvolution provides a significant progression in data analysis software, because library search tools alone fail to positively identify compounds when ≥2 coelute (12, 13).

Recent reviews illustrate the complexity of detecting pesticides in olives, apples, honey, and other foods (14–22). Essential oils from fruit are used to provide flavor and aroma for foods, beverages, and other consumer products. Lemon and orange oils, for example, are extremely complex mixtures, with each containing hundreds of organic compounds. Because chromatographic profiles change with the geographic origin of the oils, mass spectral deconvolution offers the best means of providing quantitative identification, when compared with other chemometric techniques.

This paper reports the quantitative deconvolution of lemon oil fortified with 27 pesticides, 4 surrogates, and an internal standard in 5 min. Although this analysis is probably faster than it needs to be, we wanted to demonstrate the uniqueness of the algorithms to unambiguously quantitate target compounds. The deconvolution algorithms extract ion signals, compute and compare actual versus expected relative abundances from a method or a library, and subtract, if necessary, common ion signals from interfering matrix components that elute in the same retention window as the target compound. Once spectra are deconvoluted, each scan's

Table 1. Pesticides, surrogates, and the internal standard, and the corresponding retention times, target ions, relative abundances, and relative errors used to deconvolute the mass spectrum of each target compound

Peak No.	Compound	RT, min[a]	Ion 1, *m/z*[b]	Ion 2, *m/z* (%RA)[c]	Ion 3, *m/z* (%RA)	Ion 4, *m/z* (%RA)	RE[d]
			Pesticides				
2	Dichlorvos	2.74	185	187 (33)	145 (31)		10
3	Fluorobiphenyl	2.98	172	171 (40)	170 (23)		5
5	Ethoprophos	3.40	158	200 (34)	126 (56)	139 (50)	8
6	α-BHC	3.53	181	183 (92)	219 (90)		8
7	β-BHC	3.60	181	183 (84)	219 (68)		8
8	γ-BHC	3.62	181	183 (122)	219 (97)		8
10	Disulfoton	3.66	88	97 (26)	89 (39)	142 (15)	10
11	δ-BHC	3.68	181	183 (102)	219 (86)		10
12	Parathion-methyl	3.78	109	125 (85)	263 (59)	63 (20)	5
13	Fenchlorphos	3.81	285	287 (69)	125 (58)		10
14	Heptachlor	3.82	100	272 (62)	270 (34)		10
15	Chlorpyrifos	3.89	199	314 (47)	316 (33)		10
16	Aldrin	3.91	263	261 (65)	265 (65)		5
17	Heptachlor epoxide	4.01	353	355 (95)	351 (61)	357 (37)	10
18	Endosulfan I	4.11	241	239 (60)	237 (60)	265 (60)	5
19	Prothiofos	4.12	267	309 (92)	162 (101)	113 (106)	10
20	*p*,*p*′-DDE	4.15	246	318 (68)	248 (64)	316 (56)	10
22	Dieldrin	4.19	79	81 (36)	82 (36)		10
23	Endrin	4.26	263	265 (82)	279 (45)		10
24	*p*,*p*′-DDD	4.27	235	237 (63)	165 (41)		10
25	Endosulfan II	4.28	195	241 (53)	339 (23)	337 (12)	5
26	Endrin aldehyde	4.34	345	343 (65)	347 (65)		10
27	*p*,*p*′-DDT	4.39	235	237 (65)	165 (43)		10
28	Endosulfan sulfate	4.41	387	389 (64)	422 (27)	385 (55)	10
29	Methoxychlor	4.57	227	228 (20)	238 (10)		10
30	Endrin ketone	4.60	317	315 (65)	319 (65)	209 (30)	10
32	Guthion	4.74	132	160 (119)	77 (121)		3
			Surrogates				
1	Naphthalene-d_8	2.63	136	08 (8)	137 (10)		10
4	Acenaphthen-d_{10}	3.20	162	164 (106)	160 (44)	163 (21)	10
21	*p*-Terphenyl-d_{14}	4.16	244	243 (22)	245 (19)	122 (12)	10
31	Chrysene-d_{12}	4.62	240	236 (26)	241 (20)	120 (18)	10
			Internal standard				
9	Phenanthrene-d_{10}	3.65	188	189 (16)	80 (17)	184 (13)	10

[a] RT = Retention time.
[b] Main ion used to quantitate target compound.
[c] RA = Relative abundance.
[d] RE = Relative error.

Table 2. Calibration data and recovery data for fortified lemon and orange oils

	Calibration data[a]					Avg. found in lemon oil, ng/μL								
Compound	$RF_{avg.}$[b]	RSD, %[c]	DL[d]	RL[e]	r^{2f}	100[g]	50[g]	20[g]	10[g]	5[g]	2[g]	1[g]	r^2	Recovery from orange oil, %
Dichlorvos[h]	0.084	17	2	—	0.9992	110	54	24	12	6	NI[i]	—	0.9991	99
Fluorobiphenyl	0.636	4	1	—	0.9991	105	54	22	11	6	2	1	0.9996	—
Ethoprophos[h]	0.112	16	2	—	0.9999	130	59	22	13	5	3	2	0.9967	85
α-BHC[h]	0.097	12	1	—	0.9999	100	52	21	11	6	2	1	0.9993	96
β-BHC[h]	0.089	11	1	—	0.9999	97	50	20	11	5	2	1	0.9995	94
γ-BHC[h]	0.073	14	1	—	0.9997	98	52	21	12	6	2	1	0.9982	94
Disulfoton	0.331	3	1	—	0.9999	100	56	23	12	6	3	NI	0.9945	—
δ-BHC[h]	0.076	10	1	—	0.9999	95	51	23	10	5	3	2	0.9965	108
Parathion methyl[h]	0.160	18	2	1	0.9997	110	54	22	10	5	3	2	0.9996	114
Fenchlorphos	0.213	10	1	—	0.9999	120	62	27	16	7	4	NI	0.9971	—
Heptachlor	0.137	11	2	1	0.9999	110	56	25	12	7	4	3	0.9980	—
Chlorpyrifos[h]	0.085	15	2	—	0.9997	120	62	26	14	7	3	2	0.9990	55
Aldrin	0.052	12	2	—	0.9997	100	52	22	11	5	2	—	0.9990	—
Heptachlor epoxide	0.048	12	2	—	0.9999	97	48	20	10	5	2	—	0.9999	—
Endosulfan I	0.037	13	5	–	0.9999	105	48	24	12	5	—	—	0.9954	—
Prothiofos[h]	0.068	18	2	—	0.9997	120	60	21	12	5	2	—	0.9991	89
p,p′-DDE[h]	0.156	9	1	—	0.9996	99	50	20	11	6	2	1	0.9997	92
p-Terphenyl-d_{14}[j]	0.709	11	2	1	0.9997	120	54	21	10	6	3	2	0.9965	—
Dieldrin	0.142	4	2	1	0.9999	98	50	20	10	5	NI	NI	0.9998	—
Endrin	0.022	13	5	—	0.9997	110	54	22	12	6	—	—	0.9997	—
p,p′-DDD[h]	0.317	14	1	—	0.9996	110	54	23	13	7	4	2	0.9987	92
Endosulfan II	0.026	12	20	—	0.9996	100	48	22	—	—	—	—	0.9976	—
Endrin aldehyde	0.021	20	5	—	0.9992	110	54	22	10	5	—	—	0.9997	—
p,p′-DDT	0.229	18	1	—	0.9993	110	55	22	13	6	3	1	0.9995	—
Endosulfan sulfate	0.019	11	20	—	0.9993	104	49	16	—	—	—	—	0.9931	—
Methoxychlor	0.352	11	2	—	0.9995	94	52	20	10	5	2	—	0.9968	—
Endrin ketone	0.038	15	5	—	0.9996	105	49	20	10	5	—	—	0.9985	—
Guthion	0.130	20	5	2	0.9993	105	54	24	14	8	3	—	0.9951	—
Avg.						106	53	22	12	6	3	2		
RSD, %						9	7	10	13	15	27	40		

Table 2. (*continued*)

Compound	Calibration data[a]					Avg. found in lemon oil, ng/μL							Recovery from orange oil, %
	$RF_{avg.}$[b]	RSD, %[c]	DL[d]	RL[e]	r^2[f]	100[g]	50[g]	20[g]	0[g]	5[g]	2[g]	1[g]	r^2	
Surrogate, 20 ng														
Naphthalene-d_8	1.161[k]	4												104[k]
Acenaphthen-d_{10}	0.536[k]	2												95[k]
Chrysene-d_{12}	0.712[k]	4												109[k]

[a] For 1–100 ng/μL.
[b] $RF_{avg.}$ = Average response factor.
[c] RSD = Relative standard deviation.
[d] DL = Detection limit.
[e] RL = Reporting limit; when no concentration is reported, RL = DL.
[f] r^2 = Correlation coefficient.
[g] Added, ng/μL.
[h] Pesticide also added to orange oil.
[i] NI = Ion signals were not acquired by the instrument.
[j] Surrogate.
[k] n = 14.

variability is compared with the variabilities of all other scans and with the relative error (RE) criterion set by the analyst. If the scan-to-scan variability is <RE and all deconvoluted and reconstructed ion signals for a given compound comaximize, the compound is considered present in the sample.

In contrast with target compound analysis, in which retention times are used as a primary filter, we correctly identified as unknowns 112 pesticides added to orange oil on the basis of data from a library that contained MS information only. No false positives or negatives were found after QC of the sample file. Based on the visual depiction of the ion currents in the peak, the QC process literally takes minutes, rather than hours, to perform. More than 750 compounds were screened, whose structure and composition differed greatly in size, shape, functionality, and fragmentation pattern. Because pesticides are known to cause adverse health effects (23–28), governments continue to increase the number of pesticides regulated; deconvolution can play a key role in simplifying sample pretreatment procedures that use GC/MS.

Experimental

Standard solutions of organophosphorus and chlorinated pesticides, surrogates (naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{12}, and *p*-terphenyl-d_{14}), and the internal standard (phenanthrene-d_{10}) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Calibration standards were prepared in toluene to contain pesticides and *p*-terphenyl-d_{14} at 100, 50, 20, 10, 5, 2, and 1 ng/μL. Also contained in the standards at 20 ng/μL were the other 3 surrogates and the internal standard. Lemon oil was fortified with these standard solutions. In addition, an orange oil extract was prepared by adding 112 pesticides at 2 ng/μL per pesticide.

A Gerstel (Mülheim an der Ruhr, Germany) Modular Accelerated Column Heater (MACH) and an Agilent Technologies Model 6890/5975 gas chromatograph/mass spectrometer were used for fast GC/MS. The MACH module consisted of a fused silica capillary column; in this case a Restek (Bellefonte, PA) column, Rtx-5, 10 m × 0.18 mm id with 0.20 μm film thickness, was wrapped with insulated heating and temperature sensor wires. Heat-conducting aluminum foil was wrapped around the column package to help hold it tightly together and to ensure efficient energy transfer. The MACH module was mounted on the oven door of the gas chromatograph and heated independently. The GC oven, which was set at 300°C, contained the transfer lines from the injection port to the MACH and from the MACH to the mass spectrometer. Gerstel software controlled the MACH's temperature program and was integrated into the Agilent Technologies GC/MS operating software.

All lemon oil samples were analyzed by using a 1 μL injection volume at a split ratio of 30:1 through a PTV inlet (CIS4; Gerstel). The initial injector temperature was 60°C, which was rapidly increased to 320°C at 12°C/s and held isothermally for 5 min after injection. The temperature of the MACH was programmed as follows: 40°C for 1 min, increased to 300°C at 80°C/min, then held isothermally for

10 min. Helium was used as the carrier gas under constant flow conditions of 0.7 mL/min. The mass spectrometer was operated in the scan mode from 35 to 550 *m/z* at the rate of 9.7 scans/s. The solvent delay time was 2.0 min.

In contrast, a 2 μL splitless injection at 250°C was used for the orange oil. A 30 m DB-5MS (Agilent, Little Falls, DE) column, with 0.25 mm id and 0.25 μm film thickness, was held at 50°C for 1 min; the temperature was increased to 200°C at 40°C/min, then to 300°C at 20°C/min. The mass spectrometer was scanned 3.3 times/s from 50 to 450 *m/z*. The solvent delay time was 2.5 min.

The Ion Fingerprint Detection™ deconvolution algorithms, developed at Tufts University (Medford, MA) and embedded by Ion Signature Technology, Inc. (North Smithfield, RI) into its quantitative deconvolution data analysis software, were used in this investigation. The algorithmic details and a description of how the deconvolution process works can be found elsewhere (1).

Results and Discussion

Our first objective was to demonstrate the quality of the quantitative data produced by the Ion Signature deconvolution data analysis software for target compound analysis in essential oils. A second objective was to show how the Ion Fingerprint algorithms can be used to identify unknowns in a library containing a wide range of functionalized compounds. A third objective was to establish full-scan MS, with deconvolution, as the preferred approach for analyzing pesticides in complex mixtures.

Target Compound Analysis

Typical TIC chromatograms for lemon oil are shown in Figure 1 under conventional (30 min) and fast (5 min) GC/MS operating conditions. Shown also is the reconstructed ion current (RIC) chromatogram after deconvolution for the fast analysis. Table 1 lists the target compounds and their corresponding RIC peak numbers, retention times, main and qualifier ions, corresponding relative abundances, and acceptable scan-to-scan RE. More than 150 peaks are distinguishable in the slower analysis, which means ≥150 compounds in lemon oil are potential matrix interferents in the fast analysis.

Instrument calibration and target compound recovery results under fast GC/MS conditions are shown in Table 2. Calibration and fortification data are based on the analyses of 100, 50, 20, 10, 5, 2, and 1 ng/μL of each pesticide and of the surrogate *p*-terphenyl-*d1*$_4$ and 20 ng/μL of each of the other surrogates. As expected, the detection limit (DL) values differed greatly by compound, from 20 ng/μL for endosulfan II to 1 ng/μL for BHC. The DL was established when the actual and expected relative abundances of the main and confirming ions were in agreement in ≥4 consecutive scans across the peak, and when the peak area was proportional to the signal for other concentrations. In contrast, we defined the reporting limit (RL) as an estimate of the concentration, when the RIC signal was at a concentration below the DL.

Rather than the traditional superimposed selected-ion extraction displays shown by conventional data analysis software, Figure 2 displays the deconvoluted RIC chromatograms obtained from the corresponding TIC chromatograms. The shaded bars provide a more visual display of the quality of the match between the expected and actual relative abundances (29). The first bar in each scan is set by software to be the main (or quantitative) ion, with confirming ions in as second and third bars in each scan. In this example, *m/z* 132, 160, and 77 are the main and confirmation ions, with the latter two ions scaled to the main ion. When the bars at a given scan reach the same approximate height, the differences between actual and expected ion abundances are small. When the differences in each of the peak scan histograms are small, the mathematical distance of one scan to the next will be close to 0. Similarly, the scan-to-scan variability will also be close to 0. Ideally, if all confirming ions scale to exactly the same height as the main ion, all peak scans will be exactly the same, with ion abundance ratios that exactly match the information in the method or library. In this example, each peak scan reveals a histogram of shaded bars flat across the top, with bar heights proportional to the number of fragment ions detected. If this is the case, the RE at each scan and the distance any scan is from any other peak scan are equal to 0.

Similarly, the correlation coefficient of a line, for example, a calibration curve, provides an indication of how well each point falls on the line, when compared with all other calibration points (based on the statistics of the line). If $r^2 = 1$, we know that all points fall on the line and that the distance of any 1 point from the line is 0, because the correlation coefficient detects only the linear dependencies between the 2 variables. If $r^2 = 0.85$, we know that the opposite is true and that the likelihood of points falling on the line is poor.

On the basis of the above discussion, 16 scans meet the criterion RE ≤3 for Guthion in RIC chromatogram c in Figure 2. Visually, because the main and confirming ion bars are about the same height for the first and last scans, their RE values should be close to 0 (0.13 and 0.14, respectively). Conversely, the RE for scan 4 is 1.79. Although it is less than the criterion set to determine compound presence, it is also the least similar when compared with RE values for other scans in the peak, whose maximum RE = 0.89. Identification is made when ≥3 scans are observed in the retention window. Although the RIC chromatograms b (2 ng) and c (5 ng) exceed the 3-scan minimum used to ensure compound identity, the peak area obtained from RIC chromatogram b was not proportional to the peak areas in RIC chromatogram c and those of other standards whose signals make up the calibration curve. Thus, ion current signals from the 2 and 5 ng standards established the RL and DL, respectively.

Also included in Table 2 for each compound are the average response factor ($RF_{avg.}$), relative standard deviation (RSD), and correlation coefficient (r^2) for the calibration line. The RSD was excellent for the $RF_{avg.}$ for each surrogate,

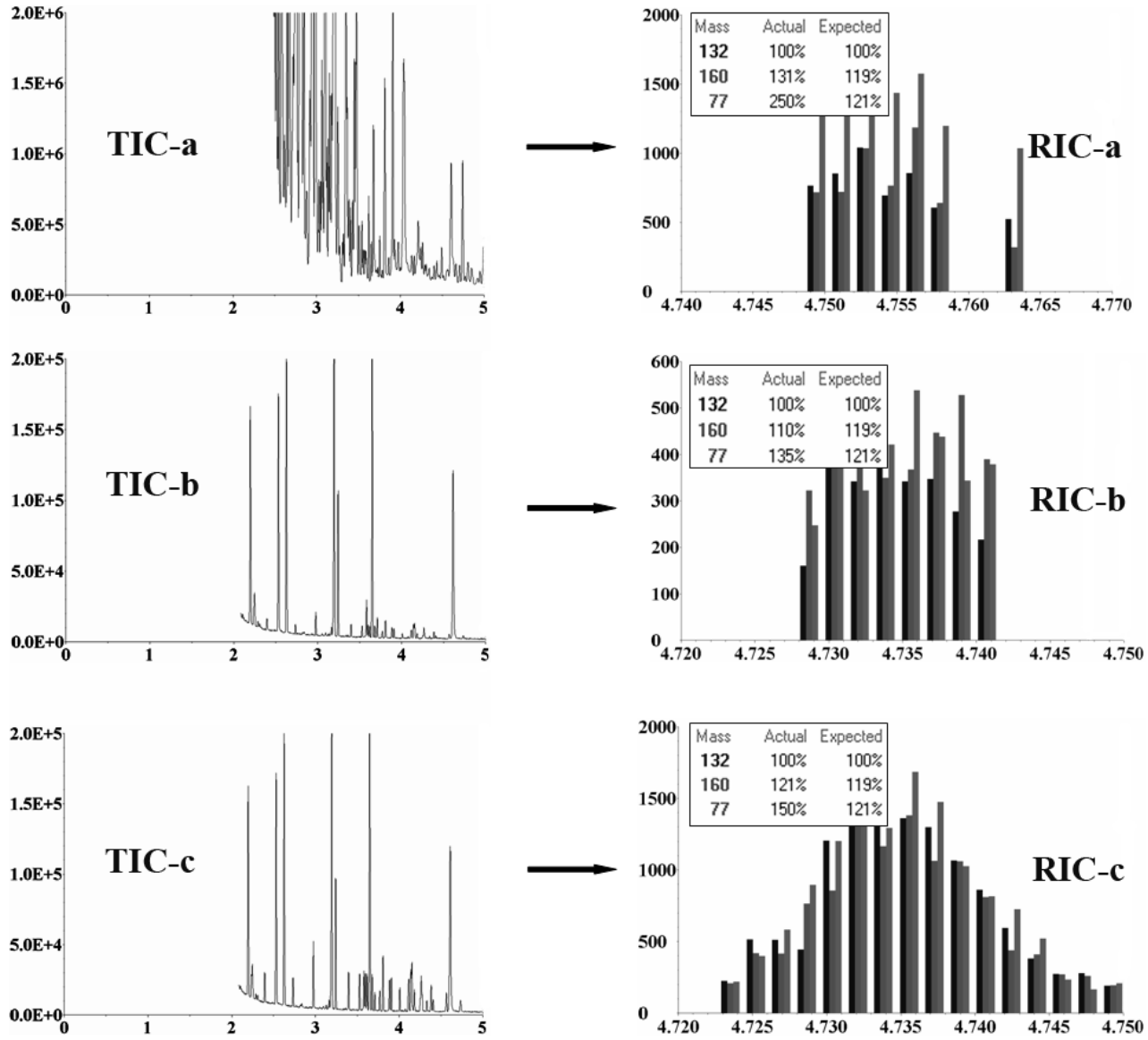

Figure 2. Total ion current (TIC) and reconstructed ion current (RIC) chromatograms for (a) pesticides at 2 ng/μL in lemon oil, (b) Guthion standard at 2 ng/μL, and (c) Guthion standard at 5 ng/μL. The main ion (*m*/*z* 132) and 2 confirming ions (*m*/*z* 160 and 77) are depicted in bar format in the RIC chromatograms, respectively. Actual versus expected ion ratios are obtained from scans at the peak maxima.

whose addition, except for *p*-terphenyl-d_{14}, was at constant concentration compared with those of the pesticides. Although the results for the pesticides and *p*-terphenyl-d_{14} are somewhat poorer, the data are well within the 25% RSD criterion. The recoveries of pesticides and surrogates from lemon oil are in remarkable agreement with the calibration data, except those for dichlorvos, disulfoton, fenchlorphos, and dieldrin, whose signals on cursory inspection of the integration report appear lost in lemon oil noise at low concentrations. On closer examination of the compound details window in the data analysis software, ion signals for these pesticides were not acquired by the instrument (*see* the corresponding calibration and recovery concentrations).

Figure 2a shows the RIC chromatogram for Guthion at 2 ng/μL lemon oil. Despite the retention time shift due to mass effects through the column, 6 consecutive scans were observed. The algorithms minimize the common ion effect at *m*/*z* 77, but they still cannot account for the increased ion current from the matrix at the 7th and 8th scans when the RE was set to 3. Hence, ion current values from scans 7 and 8 were not included in the peak area count. By raising the RE to 5, the missing scans appeared. The relative abundances for

Table 3. Pesticides, in the library, corresponding ions, and relative abundances

No.	Compound	Ion 1, *m/z*	Ion 2, *m/z* (%RA)[a]	Ion 3, *m/z* (%RA)	Ion 4, *m/z* (%RA)	Ion 5, *m/z* (%RA)	Recovery, %[b]
1	Acephate	136	94 (48)	95 (25)	96 (15)	125 (15)	108
2	Acetamiprid	152	126 (64)	166 (38)	141 (20)	181 (20)	55
3	Acrinathrin	192	181 (25)	208 (20)	289 (5)	120 (40)	99
4	Bendiocarb	151	166 (42)	223 (15)	126 (45)	152 (10)	94
5	Benfuresate	387	402 (40)	344 (20)	388 (30)	371 (15)	105
6	Bitertanol-1	170	168 (21)	171 (17)	112 (15)	141 (10)	145
7	Bitertanol-2	170	168 (21)	171 (17)	112 (15)	141 (10)	39
8	BPMC (fenobucarb)	121	150 (30)	107 (10)	151 (5)	135 (5)	103
9	Butylate	146	174 (57)	217 (24)	156 (50)	75 (25)	113
10	Cadusafos	159	88 (45)	158 (69)	187 (10)	189 (10)	108
11	Captafol	79	183 (8)	276 (3)	107 (15)	149 (10)	56
12	Captan	79	117 (13)	149 (22)	119 (20)	107 (20)	114
13	Chinomethionat	234	116 (68)	206 (112)	174 (40)	173 (25)	102
14	Chlorobenzilate	251	139 (88)	141 (30)	253 (66)	111 (35)	111
15	CVP (chlorfenvinphos)-E	267	323 (38)	325 (43)	295 (30)	297 (20)	92
16	CVP (chlorfenvinphos)-Z	267	323 (63)	325 (41)	295 (30)	297 (20)	94
17	Cyfluthrin-1	163	165 (66)	206 (56)	226 (25)	199 (30)	108
18	Cyfluthrin-2	163	165 (65)	226 (50)	206 (40)	199 (30)	127
19	Cyfluthrin-3,4	163	165 (66)	226 (50)	206 (40)	227 (10)	90
20	Cyhalothrin-1	181	197 (75)	208 (60)	141 (25)	77 (25)	99
21	Cyhalothrin-2	181	197 (70)	208 (59)	141 (25)	77 (25)	78
22	Cypermethrin-1	163	165 (64)	181 (90)	180 (20)	127 (30)	83
23	Cypermethrin-2	163	165 (60)	181 (50)	208 (15)	209 (15)	96
24	Cypermethrin-3,4	163	165 (60)	181 (69)	209 (10)	152 (10)	117
25	Cyproconazole	222	139 (60)	224 (40)	179 (70)	180 (10)	136
26	Deltamethrin	181	253 (58)	255 (28)	251 (30)	172 (30)	111
27	Diazinon	179	137 (98)	152 (70)	199 (50)	304 (60)	104
28	Dichlofluanid	123	167 (35)	224 (30)	226 (20)	332 (5)	121
29	Dicofol	251	139 (90)	253 (60)	111 (30)	141 (30)	121
30	Diethofenccarb	267	196 (65)	225 (108)	296 (110)	297 (90)	110
31	Difenoconazole-1	265	267 (65)	323 (90)	324 (60)	325 (15)	139
32	Difenoconazole-2	265	267 (65)	323 (90)	324 (60)	325 (15)	141
33	Dimethipin	54	118 (22)	210 (5)	151 (5)	103 (5)	112
34	Dimethylvinphos	295	297 (65)	299 (15)	229 (10)	243 (15)	84
35	Edifenphos	109	173 (70)	310 (30)	201 (20)	218 (10)	112
36	EPN (Santox)	157	169 (66)	185 (30)	141 (30)	323 (15)	84
37	EPTC (Eptam)	128	86 (82)	132 (25)	160 (10)	189 (25)	79
38	Esprocarb	222	91 (185)	162 (57)	196 (80)	197 (70)	109
39	Ethiofencarb	107	108 (13)	168 (30)	139 (5)	225 (5)	102
40	Etrimfos	292	263 (12)	277 (33)	181 (85)	153 (70)	93
41	Fenapanil	139	219 (57)	251 (48)	253 (30)	330 (30)	80
42	Fensulfothion	293	308 (40)	141 (40)	97 (40)	125 (30)	103
43	Fenvalerate-1	125	167 (78)	225 (67)	419 (50)	169 (40)	99
44	Fenvalerate-2	125	167 (78)	225 (67)	419 (50)	169 (40)	116
45	Flucythrinate-1	199	157 (68)	181 (28)	184 (20)	107 (25)	78

Table 3. (*continued*)

No.	Compound	Ion 1, *m/z*	Ion 2, *m/z* (%RA)[a]	Ion 3, *m/z* (%RA)	Ion 4, *m/z* (%RA)	Ion 5, *m/z* (%RA)	Recovery, %[b]
46	Flucythrinate-2	199	157 (88)	181 (41)	184 (33)	107 (44)	132
47	Flusilazole	233	234 (19)	315 (10)	206 (35)	220 (10)	82
48	Flutolanil	173	145 (40)	174 (10)	281 (26)	323 (15)	108
49	Fluvalinate-1	250	181 (28)	252 (34)	251 (15)	180 (6)	76
50	Fluvalinate-2	250	181 (26)	252 (38)	251 (12)	180 (6)	73
51	Fosthiazate-1	252	251 (60)	236 (5)	264 (5)	281 (10)	116
52	Fosthiazate-2	252	251 (60)	236 (5)	264 (5)	281 (9)	118
53	Halfenprox	263	183 (51)	265 (99)	185 (30)	415 (5)	115
54	Imibenconazole	249	187 (62)	284 (33)	202 (38)	250 (35)	
55	Ci-IPC (chlorpropham)	127	213 (50)	154 (40)	171 (40)	129 (30)	121
56	Iprodione	56	58 (60)	187 (26)	314 (40)	316 (20)	
57	Isofenphos	213	58 (220)	255 (36)	121 (30)	345 (10)	109
58	Isofenphos P=O	229	120 (18)	201 (72)	121 (15)	200 (15)	139
59	Lenacil	153	154 (9)	152 (5)	136 (5)	234 (2)	84
60	Malathion	125	173 (90)	93 (90)	127 (85)	158 (40)	116
61	Mefenacet	192	120 (38)	148 (24)	105 (10)	106 (10)	84
62	MEP (fenitrothion)	277	125 (85)	109 (70)	260 (60)	278 (10)	121
63	Mepronil	269	119 (481)	210 (23)	227 (20)	270 (20)	84
64	Methamidophos	141	94 (209)	95 (138)	111 (15)	126 (15)	81
65	Methiocarb	168	153 (63)	169 (13)	154 (12)	225 (12)	96
66	Metolachlor	162	211 (10)	238 (58)	163 (12)	240 (15)	108
67	MIPC (isoprocarb)	121	136 (40)	122 (10)	137 (5)	107 (5)	109
68	MPP (fenthion)	278	169 (19)	279 (13)	125 (36)	109 (32)	97
69	Myclobutanil	179	150 (68)	245 (22)	181 (35)	152 (25)	59
70	NAC (carbaryl)	144	115 (43)	145 (12)	116 (26)	127 (3)	127
71	Paclobutrazol	236	167 (24)	238 (33)	244 (30)	169 (10)	83
72	PAP (phenthoate)	274	135 (26)	246 (34)	121 (65)	125 (65)	94
73	Parathion	291	137 (40)	139 (40)	155 (30)	235 (25)	101
74	Pendimethalin	252	162 (22)	191 (13)	281 (15)	253 (15)	84
75	Permethrin-1	183	163 (27)	165 (20)	184 (15)	127 (10)	95
76	Permethrin-2	183	163 (27)	184 (15)	165 (20)	127 (10)	92
77	Phosalone	182	184 (33)	367 (27)	154 (27)	153 (17)	82
78	Pirimicarb	166	72 (80)	238 (23)	167 (10)	138 (8)	112
79	Pirimiphos-methyl	290	276 (77)	305 (79)	125 (50)	233 (45)	107
80	Pretilachlor	238	202 (55)	240 (33)	242 (50)	262 (50)	90
81	Propiconazole-1	259	173 (90)	175 (65)	261 (66)	191 (40)	134
82	Propiconazole-2	173	175 (60)	259 (74)	261 (43)	128 (33)	105
83	Pyraclofos	360	139 (90)	138 (70)	194 (85)	362 (40)	78
84	Pyridaben	147	117 (30)	132 (15)	148 (13)	309 (5)	89
85	Pyrifenox-E	262	187 (45)	264 (67)	263 (50)	265 (30)	99
86	Pyrifenox-Z	262	264 (67)	263 (25)	265 (20)	294 (19)	110
87	Pyrimidifen	184	185 (12)	186 (34)	360 (4)	183 (3)	86
88	Pyriproxyfen	136	186 (3)	226 (7)	137 (3)	115 (10)	87
89	Quinalphos	146	157 (67)	156 (60)	129 (40)	298 (40)	110

Table 3. (*continued*)

No.	Compound	Ion 1, *m/z*	Ion 2, *m/z* (%RA)[a]	Ion 3, *m/z* (%RA)	Ion 4, *m/z* (%RA)	Ion 5, *m/z* (%RA)	Recovery, %[b]
90	Silafluofen	179	258 (57)	286 (80)	180 (25)	287 (25)	88
91	Tebuconazole	125	250 (50)	252 (15)	127 (35)	163 (10)	103
92	Tebufenpyrad	333	276 (71)	318 (118)	320 (40)	319 (30)	109
93	Tefluthrin	124	125 (15)	135 (15)	177 (5)	197 (5)	98
94	Terbufos	231	233 (10)	125 (15)	153 (25)	288 (12)	118
95	Thenylchlor	417	432 (46)	418 (30)	431 (30)	359 (15)	85
96	Thiobencarb	100	125 (26)	257 (15)	72 (60)	89 (10)	95
97	Thiometon	88	89 (26)	125 (31)	60 (30)	61 (30)	94
98	Tolclofos-methyl	265	250 (12)	267 (40)	79 (15)	93 (15)	109
99	Triadimenol-1	112	168 (70)	128 (70)	130 (20)	70 (20)	133
100	Triadimenol-2	112	128 (50)	168 (50)	130 (15)	70 (40)	135
101	Tricyclazole	189	190 (12)	191 (7)	162 (55)	161 (30)	125

[a] RA = Relative abundance.
[b] Ratio of ion 1 extracted from fortified oil to ion 1 extracted from the standard.

these scans were within the variability of the other scans, which means that the higher acceptance threshold resulted in the integration of all peak scans. The software allows the analyst to optimize those peaks scans that should be integrated. Therefore, after calibration, the analyst should adjust the RE up or down on the basis of analyses of a few typical samples, taking care not to include peak area from the matrix that will result in overestimation of the concentration. The criterion we used for compound acceptance ensured an average calibration response factor with an RSD of ≤20%; thus, the RL concentrations were ≤DL. On the basis of this rationale, insufficient scans exist to confirm Guthion in either the 1 ppm standard or a fortified sample. For those pesticides detected between 100 and 5 ng/μL lemon oil, the RSD of the average recovery for all pesticides was ≤20%, which is excellent. The 1 and 2 ng/μL samples were also well within the criterion of an RSD of 50% at concentrations 5 times the DL. Although only 2 measurements were made at each concentration, the relative difference was <10% for all compounds detected.

Identification of Unknowns

A library of mass spectral information for >750 compounds was compiled that included polyhalogenated volatiles, biphenyls, dibenzothiophenes, and dibenzofurans; alkylated and deuterated polycyclic aromatic hydrocarbons; mercaptans; triazoles; quinoxalins; and cyano compounds. In addition to the pesticides in Table 2, the 101 pesticides in Table 3 were added to the library. As many as 5 ions per compound were used, with ions and relative abundances obtained from NIST, Wiley, or the published literature (30, 31). Unlike target compound analysis, in which ion abundance ratios are optimized on the basis of calibration curve responses, library ion ratios were used as found. Compound identification was established when the uncertainty in the relative abundance was ≤20% for each peak scan and the scan-to-scan variability was ≤5. The deconvolution algorithms did not rely on retention time data to determine compound identity.

Initially, we wanted to demonstrate that the 27 pesticides, 4 surrogates, and internal standard could be identified correctly solely on the basis of mass spectral data, with minimal false positives and negatives. All 32 compounds were correctly found in the lemon oil sample. Isomeric peak assignments were not possible, as expected, in the absence of compound-specific retention time windows. Remarkably, for the 750 compounds in the library, only 1 false positive was observed. This occurred in the analysis of the 100 ppm lemon oil sample, for which 3 scans met the criteria for identification of dichlofluanid. At lower fortification concentrations, dichlofluanid was not detected because of the low relative abundance of its ions, which was <10% of the relative abundance of the base ion in the 100 ppm sample.

To further investigate how well the deconvolution algorithms worked, we prepared a more chemically diverse mixture of pesticides and added them to orange oil. The extract contained 2 ng/μL of each of the pesticides in Tables 2 and 3 for a total of 112 compounds, with 80% of them appearing within the unresolved chromatogram. Except for *p,p′*-DDT, which was identified at the same elution time as *p,p′*-DDD, 110 pesticides in the orange oil were identified correctly. No other compound in the library was falsely found in the sample, including the other 16 pesticides in the database. The algorithmic detection of *p,p′*-DDT should not be considered a false positive, because the molecular structure of the 2 compounds differs only by the addition of a chlorine atom on the ethane moiety, which is located between the 2 *p*-chlorophenyl groups. Under electron impact ionization

conditions and at unit instrument resolution, the resulting mass fragmentation patterns for *p,p′*-DDD and *p,p′*-DDT are the same. No ion current was acquired by the mass spectrometer for the remaining 2 compounds, imibenconazole and iprodione, in either the standard or fortified orange oil samples at the injection level of 4 ng per compound. The inability to detect these compounds is due to their inherent instability under electron impact conditions, rather than to failure of the algorithms to identify these 2 pesticides. Only 1 compound fell outside the recovery criterion of 100 ± 50%: bitertanol-2 with a recovery of 39%. Tables 2 and 3 list the recovery for each pesticide added to orange oil. The average recovery of the 110 pesticides was 101 ± 19%.

These findings demonstrate that full-scan MS, or selected-ion monitoring (SIM) MS when searching for a more limited compound set, with spectral deconvolution, offers the best opportunity to identify target compounds and unknowns in complex mixtures. We showed that a minimum of 3 ions per compound are needed to take full advantage of the algorithms. With the advent of large-volume injection technology, full-scan MS can compete with electron capture detection (ECD) or single-ion SIM detection to meet regulatory-driven limits without the need to confirm compound identity by 2-dimensional GC/ECD using 2 dissimilar stationary phases. Finally, we showed that the deconvolution algorithms provide an excellent means to meet increasing sample analysis demands for pesticide screening under extremely fast GC conditions. The Ion Signature software makes quantitative detection of target compounds and unknowns possible without extensive sample cleanup before analysis.

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