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Target GC-MS Analysis using Accelerated Column Heating and Interactive Deconvolution Software

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Abstract
In diverse fields ranging from environmental samples to complex flavor preparations, high performance, high resolution capillary gas chromatography/mass spectrometry, GC-MS, has become the preferred technique for identifying and quantifying target compounds at trace levels. Many of these samples are characterized by very complex matrices, which provide a substantial challenge for contemporary capillary GC-MS. This situation is now even more complicated by the current trend towards faster GC analysis using reduced diameter columns, accelerated column heating, or a combination of both.

In complex mixture analysis, new approaches and techniques are needed in order to extract the required data from the total ion current chromatogram. Although two-dimensional GC can chromatographically deconvolve complex samples, it can be a relatively expensive and time-consuming process that may not always be the best choice for routine analysis. Retention time locked GC-MS techniques, with dedicated libraries, work well when the

matrix is not too complex. As the complexity of the sample increases, however, a limit is reached beyond which powerful spectral deconvolution algorithms are needed to extract component spectra from complex spectral background.

In this presentation, interactive mass spectrometry deconvolution software (IST for GC-MS) is used to determine the presence of trace pesticide levels in a complex essential oil. The oil is injected without sample preparation and the chromatography is run under accelerated heating conditions at either 40°C/min or 120°C/min to give total run-times of between 5 min and 10 min. The IFD algorithms, the core technology, extend measurement sensitivity by deconvoluting the spectral fingerprint of the target compound from the random nature of instrumental noise. Method detection limits are also improved as the target compound fingerprint is separated from the random "sample-to-sample" variation of chemical background and spectral noise. In this way reporting limits approach method detection limits, which increases measurement precision and robustness. The software has unique and powerful interactive software features for QC validation of target compounds. The simple visual presentation and table format of the scan-to-scan data fit allows the analyst to judge whether the compound is truly present in the sample.

These considerations are true for conventional GC run-times. When fast GC conditions are applied the IFD algorithms become even more useful in extending measurement sensitivity, selectivity, accuracy, precision and robustness of the data produced.

Introduction

Food safety is an essential component of consumer protection. Countries in Asia, Europe, and the Americas routinely look for the presence of pesticides and other hazardous chemicals in food and beverage products. Japan, for example, has increased the number of chemicals it targets from less than 100 to more than 1000 organic pollutants over the last few years.

The presence or absence of key constituents is also an important component of product quality as these compounds relate to feel, taste, and aroma.

Lemon oil, for example, is used to "spice" food, beverage, and other consumer products. The presence of pesticides is a health hazard, while quality is affected by numerous factors including lemon variety, climate, soil type, and extraction method. Product quality largely depends upon the lemon‘s origin, with safety dependent on soil type, proximity to combustion sources, and chemicals used to protect the plant. Lemon oil consists of hundreds of different organic compounds making the extract itself an especially complex mixture to analyze.

Much time and effort has been spent on developing technologies and methods that increase laboratory efficiency and data quality when analyzing complex mixtures.

Advancements include sample preparation automation, low-bleed high resolution gas chromatography (GC) stationary phases, and the electronic control of GC systems with mass spectrometry (MS) detectors, which now produce higher mass resolution, better sensitivity, faster scan speeds, and simultaneous operation in scan and selected ion monitoring modes; all of which yield results not attainable a decade ago.

Chemometrics employs a variety of tool box techniques that includes signal processing, detection, resolution, calibration, and curve fitting; statistics and statistical design; factor analysis and factorial design; method optimization, library searching, pattern recognition, and neural networks. Until now, these techniques have not been very successful in providing accurate information about the sample from raw data independent of the sample matrix.

Confirmation of target compounds in complex mixtures is difficult because MS fingerprints are often masked by co-eluting matrix ions. In this work, deconvolution is applied to the mass spectral output of the analysis of lemon oil.

Ion Signature quantitative deconvolution software showed that a mixture of pesticides and surrogates "spiked" into lemon oil can be analytically quantified under fast GC/MS conditions.

Experimental

Instrumentation. Analyses were performed on a GC (6890, Agilent Technologies), equipped with a Mass Selective Detector (5975, Agilent Technologies), PTV-Inlet (CIS 4, GERSTEL), an Autosampler configured for liquid injection (MPS 2, GERSTEL), and a Modular Accelerated Column Heater (MACH, GERSTEL) mounted on the oven door of the 6890 gas chromatograph (Figure 1).

The MACH device uses Low Thermal Mass (LTM) technology, which involves combining any length standard capillary GC column, an RTD based thermal measurement system, and a precision controlled heating element in a bundle over the full length of a column. The bundle is wrapped with a ceramic twine, coiled to a 5” diameter torus that is covered with a metal foil (Figure 2). The LTM technology enables fast heating (up to 1800°C per minute), fast cooling (350°C to 35°C in 2 minutes or less), as well as independent temperature control of up to 4 column modules on one GC platform.

Figure 1. Dual Column MACH Connected to 6890 GC System.

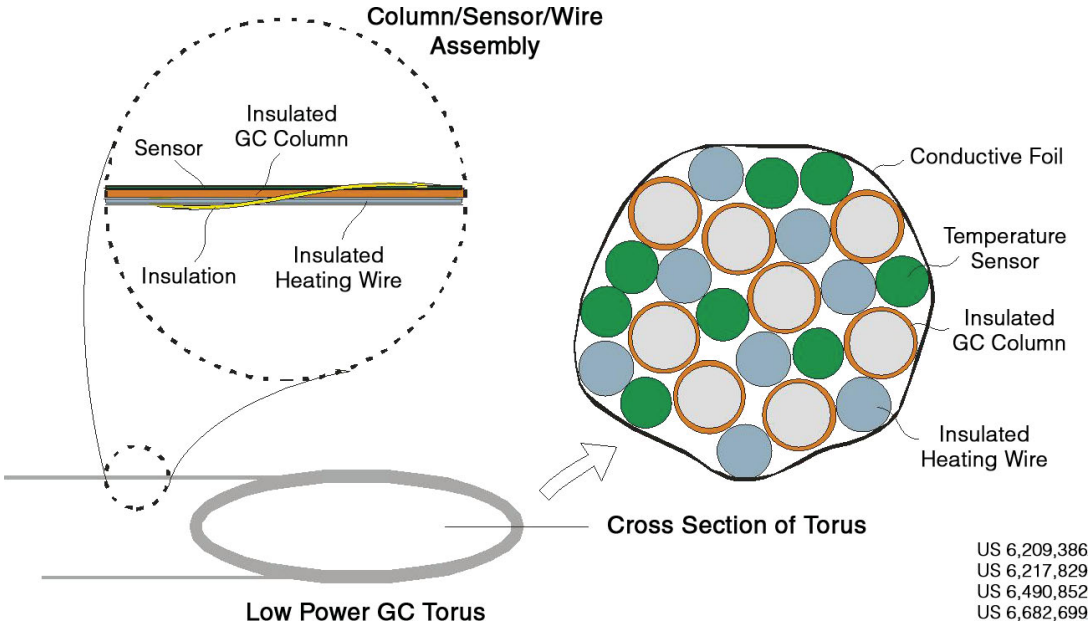

Figure 2. Low thermal mass column module diagram.

The column modules are heated outside the GC oven with transfer lines to the GC’s injector and detector going through the GC oven door. Connections to the injector and detector are identical to standard GC column connections. Programming of the temperature ramps can be performed either on a key pad on the front of the MACH door or through GERSTEL MAESTRO software integrated into the Agilent Chemstation control software.

Sample preparation. Solutions of organo-phosphorous pesticides and deuterated PAHs (as internal standards / Surrogates) were prepared in toluene and in lemon-oil, covering concentration levels of 100, 50, 20, 10, 5, 2 and 1 ng per µL toluene / lemon-oil. Table 1 lists the compounds, their retention times and mass spectral ions used to untangle the target compound signal from matrix signals.

Table 1. Target compounds used in this study.

No.	Compound	RT [min]	Quant / Qualifier Ions
1	Phenanthrene-d10	3.68	188 / 189, 184
2	1,4-Dichlorobenzene-d4	2.26	150 / 152, 115, 78
3	Napathalene-d8	2.78	136 / 108, 137
4	Dichlorvos	2.81	185 / 187, 145
5	Acenaphthene-d10	3.23	164 / 162, 160
6	Ethoprophos	3.43	158 / 97, 126, 139
7	Disulfoton	3.70	88 / 60, 89
8	Chrysene-d12	4.56	240 / 236, 120
9	Parathion-Methyl	3.81	109 / 263, 79, 93
10	Fenchlorphos	3.84	285 / 287, 125, 289
11	Chlorpyrifos	3.93	197 / 199, 314, 97
12	Mecarbam	4.04	131 / 97, 159, 125
13	Prothiofos	4.16	267 / 309, 162, 113
14	Ethion	4.31	231 / 97, 153, 125, 121
15	Guthion	4.64	132 / 160, 77, 104
16	Perylene-d10	5.36	264 / 132, 265

Analysis conditions.

Injection:	1 µL, MPS 2
PTV:	split 1:30 60°C; 12°C/s; 320°C (5 min)
GC Oven:	300°C, held for duration
MACH Module:	10 m Rtx®-5 (Restek) d_i = 0.18 mm, d_f = 0.20 µm He, constant flow, 0.7 mL/min 40°C (1 min); 80°C/min; 300°C (10 min)
MSD:	scan, 35-550 amu, 9.69 scans/s

Data analysis. Deconvolution is a mathematical technique for quickly and accurately extracting the equivalent of clean spectra from background noise or from coeluting compound peaks. Equation 1 computes the ratio between an established library and observed relative abundances to match target compound spectra with library spectra:

$$f_i(t) = \frac{R_i(t)}{L_i} A_m(t) \qquad (1)$$

L_i is established library abundance ratio

R_i (t) is observed relative abundance for the i^{th} ion ($1 \le i \le N$)

A_m (t) is observed abundance of the main ion

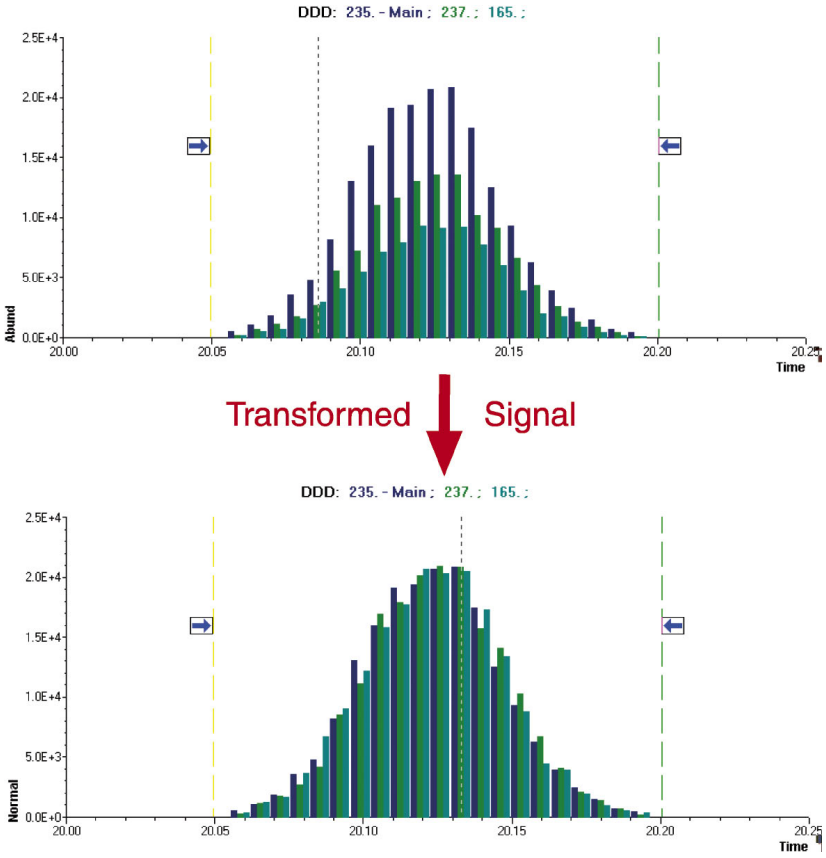

Figure 3. Transformed signal.

A total of three different algorithms are used to identify compounds in the sample (Equation 2):

$$F_1(t) = \max_{i \le N}[f_i(t)] - \min_{j \le N}[f_i(t)]$$

$$F_2(t) = \frac{\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left| f_i(t) - f_j(t) \right|}{\sum_{i=1}^{N-1} i} \qquad (2)$$

$$F_3(t) = \left| \max_{i \le N} \frac{d f_i(t)}{dt} - \min_{j \le N} \frac{d f_j(t)}{dt} \right|$$

as present in the sample and is quantified.

$$\Delta_1(t) = K^{\%} \left| \max_{i \le N} f_i(t) \right| + \Delta_0$$

$$\Delta_2(t) = \alpha K^{\%} \left| \max_{i \le N} f_i(t) \right| + \Delta_0$$

$$\Delta_3(t) = \beta K^{\%} \left| \max_{i \le N} \frac{d f_i(t)}{dt} \right| \qquad (3)$$

$K^{\%}$ - acceptable difference (%)

α, β - coefficients

Target Compounds Detected

when $F_1(t) \le \Delta_1(t)$ **and/or** $F_2(t) \le \Delta_1(t)$

The acceptable relative abundance errors are calculated with the following equations (3). When the scan-to-scan variation falls within the analyst established value for each compound, the compound is reported

The analyst can inspect the deconvoluted signal and quickly and easily determine the accuracy of the process. The compound is identified, when F1(t) = F1(t) or F1(t) = F3(t) or F2(t) = F3(t).

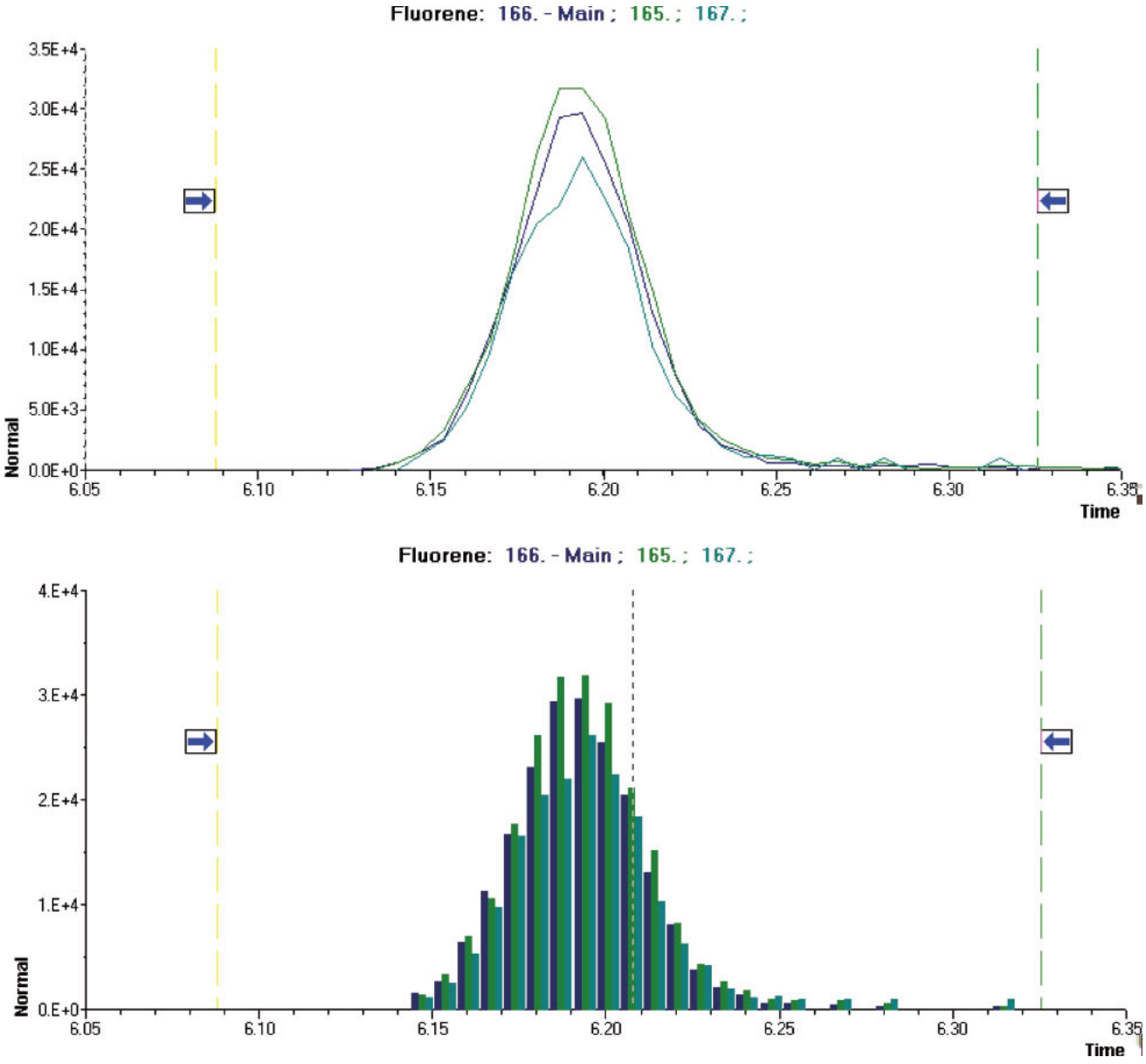

Figure 4. Compound identified.

After deconvolution, all qualifier ions are normalized to the quantifier ion and should therefore be equal in height to the quantifier ion at each scan if the compound of interest is present in the sample.

Results and Discussion

Figure 5 shows a comparison between conventional and fast analysis.

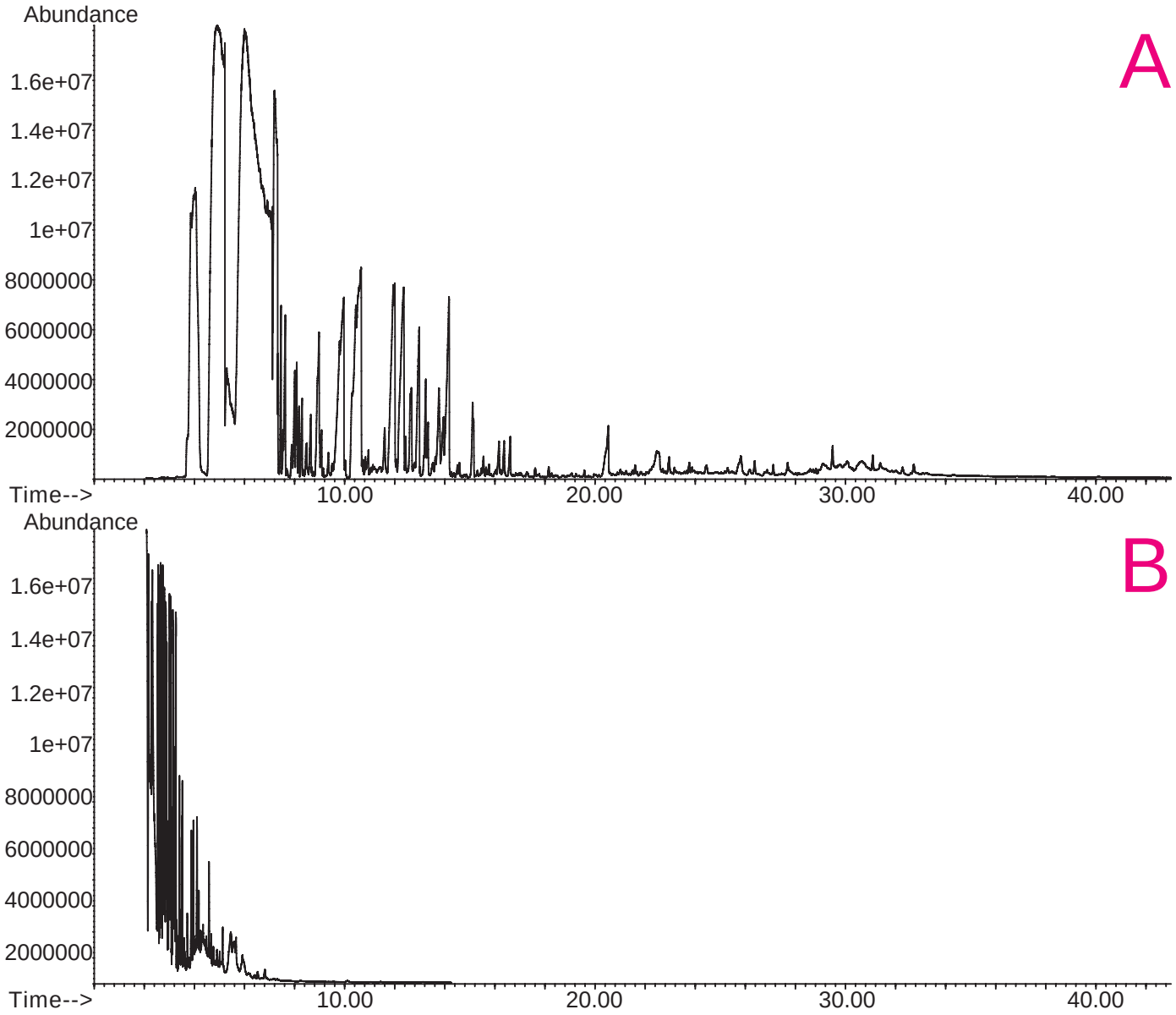

Figure 5. Chromatogram of lemon oil under conventional (A) and fast (B) run conditions.

The pesticides in the samples are deconvoluted using quadrupole mass spectrometry ion signatures. All pesticides , surrogates, and internal standards are detected and quantified in scan mode (Figure 6).

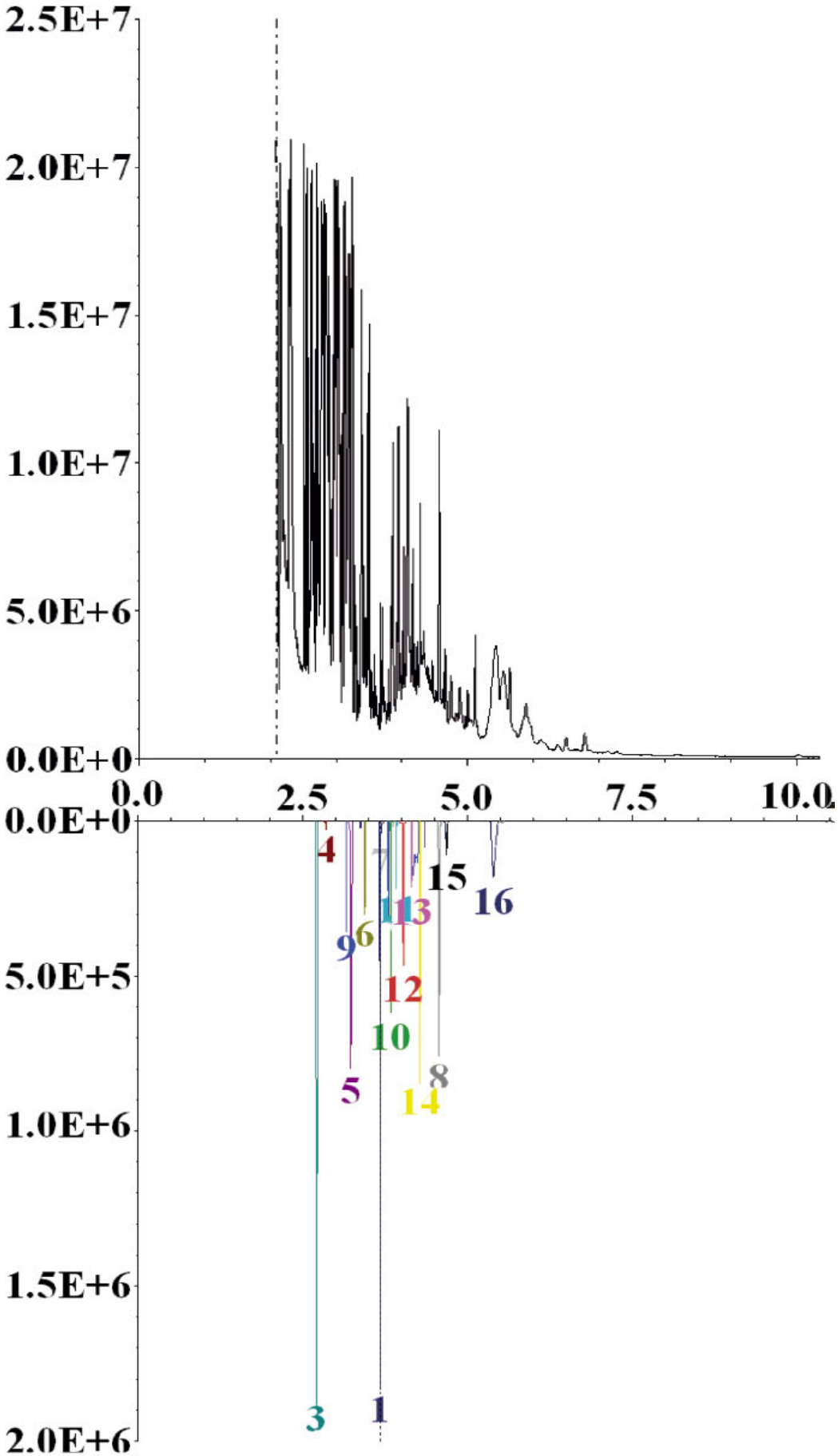

Figure 6. Total ion current chromatography (A) and reconstructed ion current chromatography (B).

The Ion Signature quantitative deconvolution software unambiguously differentiates target compound mass spectra from "chemical noise" (Figure 7).

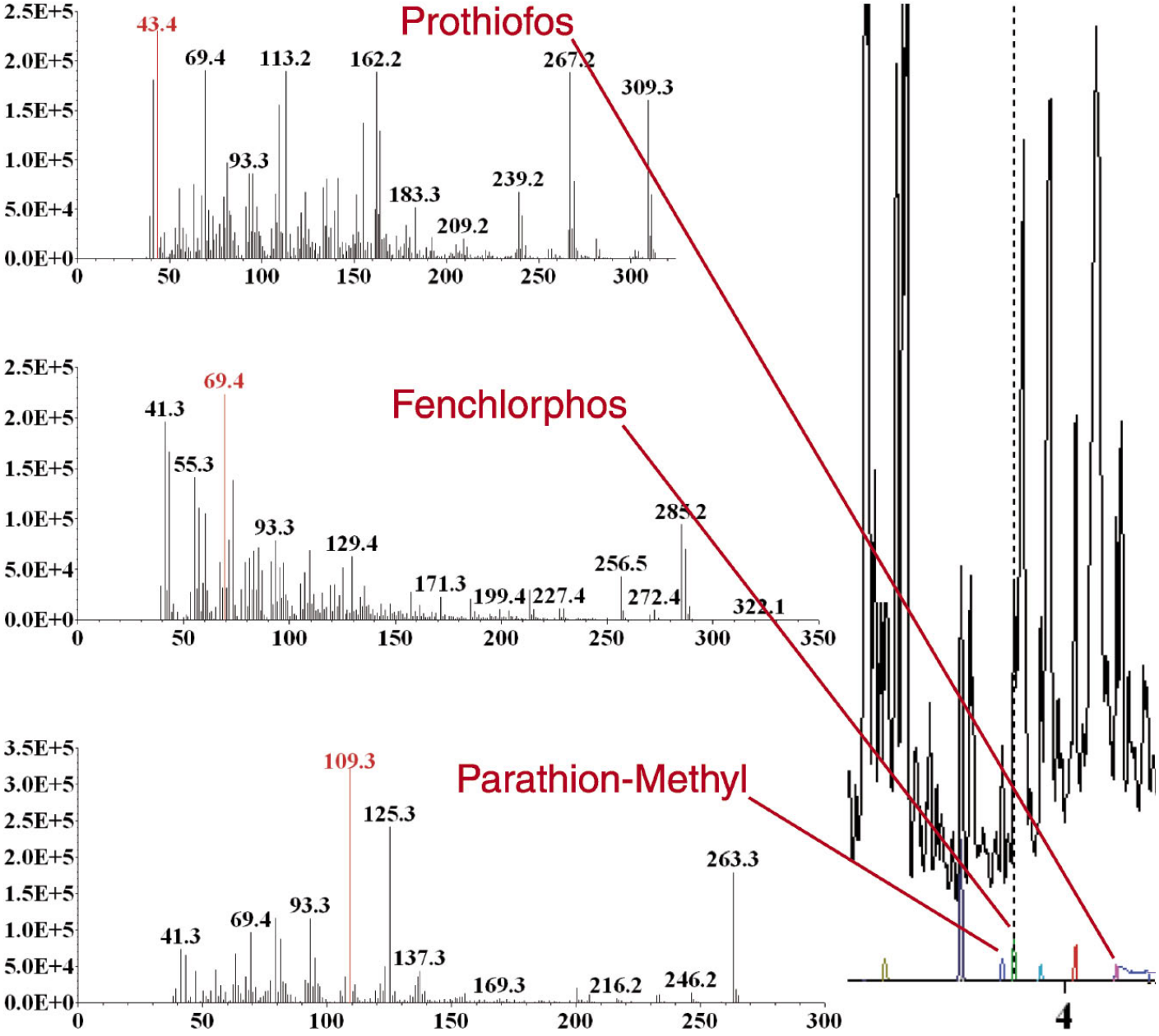

Figure 7. Target compound mass spectra differentiated from chemical noise.

The internal standard, perylene-d12, mass spectrum is buried in high concentration chemical noise as it elutes on the shoulder of a major lemon oil peak (Figure 8A).

After deconvolution, qualifier ions are normalized to the quantifier ion and shown as repetitive histograms at every scan across the peak (Figure 8C).

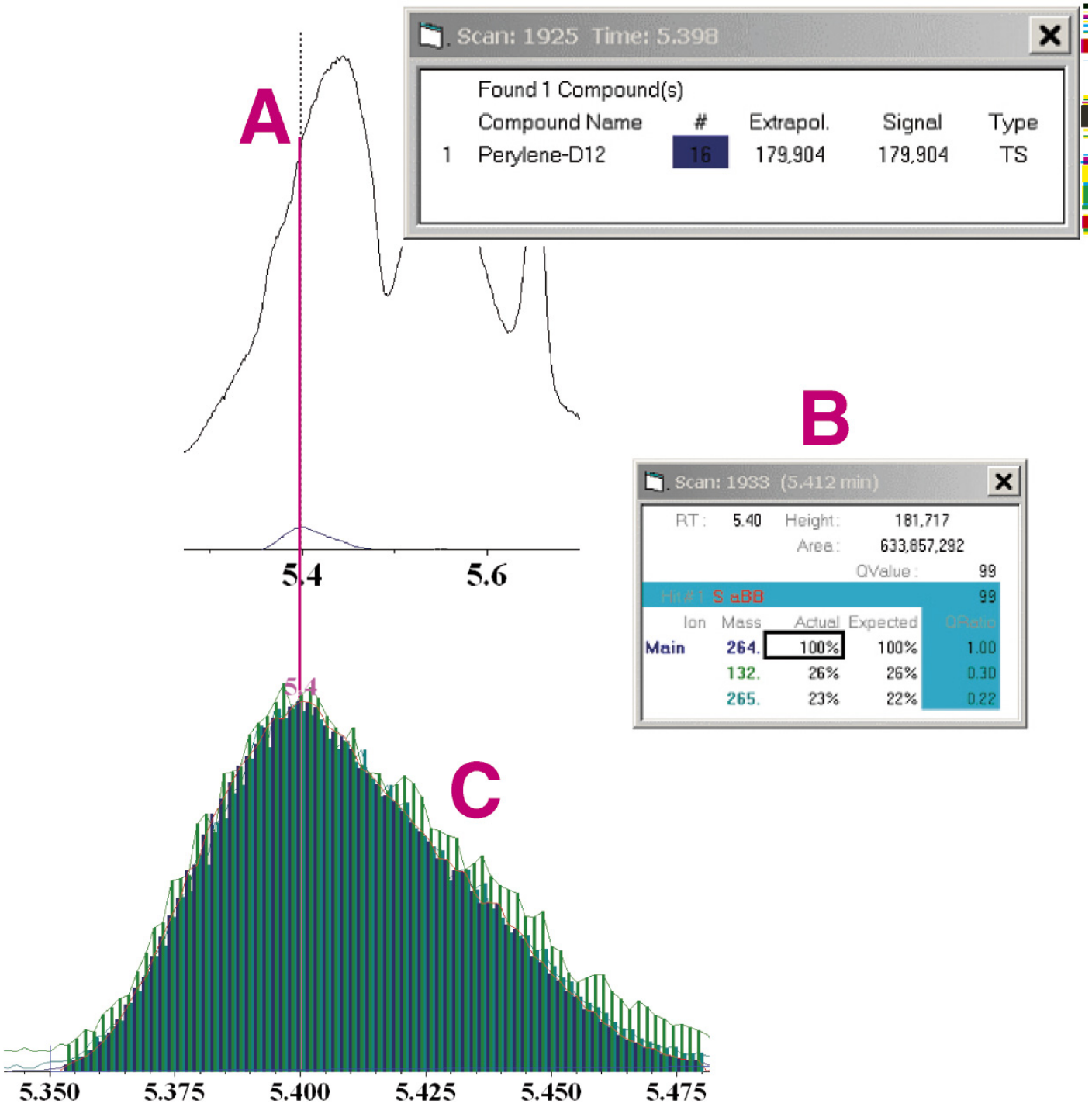

Figure 8. Perylene-d12: TIC (A), actual versus expected ion ratios (B), repetitive histogram across the peak (C).

The graph in figure 9 shows the pesticide recovery as a function of concentration.

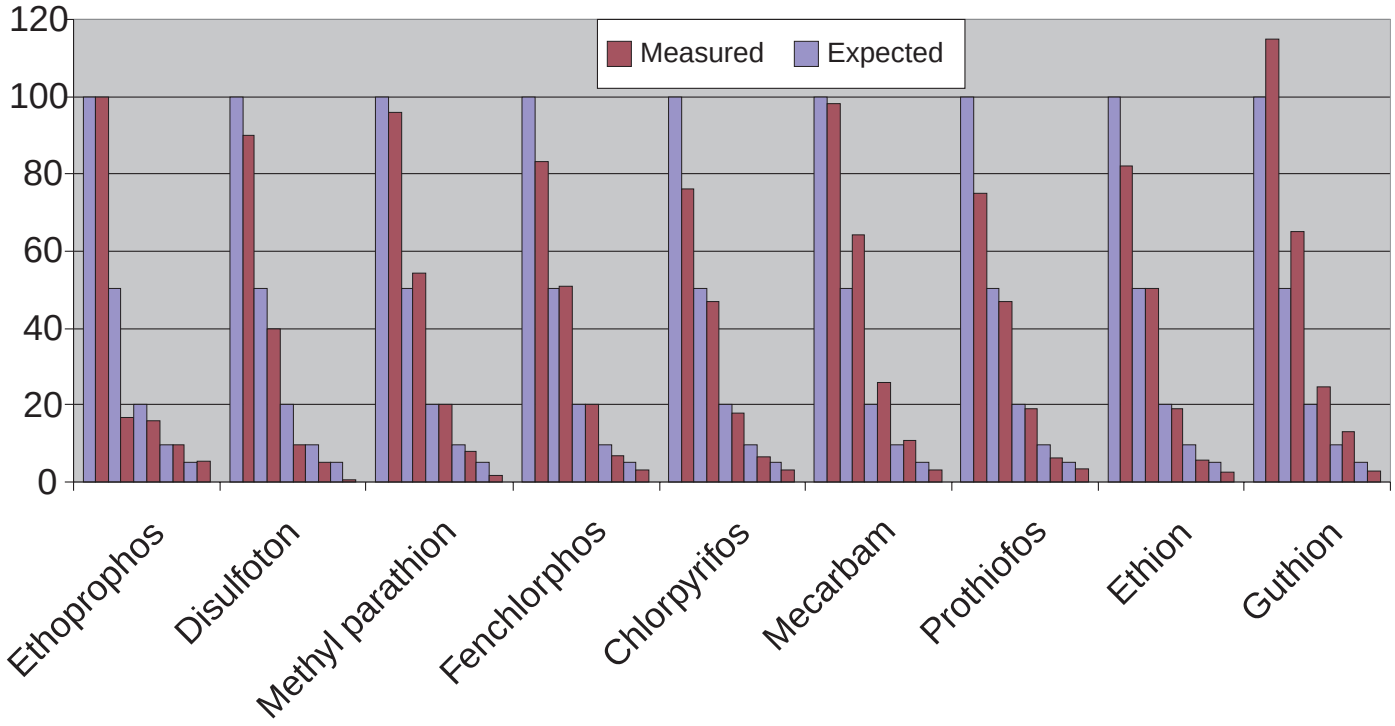

Figure 9. Pesticide recovery.

Surrogates were added to each pesticide - lemon oil sample (100, 50, 20, 10, 5, and 2 ppm) at 20 ppm concentration. The average surrogate recovery was 17 ± 6 ppm. The average measured concentration for all pesticides across the concentration range of 2 ppm to 100 ppm was within 20% of the expected, fortified, concentration.

Conclusions

The MACH‘s ability to start at a low temperature, and then rapidly heat the column for rapid separation provides the analyst with a powerful analytical tool. We showed that we could accelerate conventional methods, cutting cycle time to less then a third which can potentially triple throughput on a single instrument.

By taking advantage of these abilities and combining it with a powerful deconvolution and data analysis software, commercial environmental labs can improve throughput without sacrificing data quality.

The Ion Signature quantitative data analysis software allows the analyst to quickly inspect extracted mass ions and computed relative abundances after deconvolution. Using these interactive features the analyst can judge which compounds are present in the sample by making data quality review a simple task rather than accepting a „black box“ report.

Ion Signature successfully deconvoluted target compound spectra from matrix spectra, calculated the correct peak area, and quantified pesticides and surrogates within analytically accepted recovery ranges.

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