Application Note

Instrument: Pegasus® GC-HRT+

Enhancing of a Powerful Discovery Tool with a Novel Multi-Mode Ion Source

Key Words: Electron Ionization, Positive Chemical Ionization, Electron Capture Negative Ionization, High Resolution Time-of-Flight Mass Spectrometry

Introduction

For the past 10 years, LECO Corporation's High Resolution Time-of-Flight (GC-HRT+) mass spectrometer has aided in the advancement of forensics,[1,2] environmental science,[3-5] metabolomics,[6,7] food/flavor,[8,9] and energy[10] research. The coupling of robust and reproducible chromatography with high-resolving capabilities of the Folded Flight Path® (FFP®) mass analyzer has expedited characterization of complex samples. This was accomplished through the reduction of matrix interferences, improved automated processing, high mass accuracy and analysis of rich, comprehensive mass spectral data.

Recently, the introduction of a novel Multi-Mode Source™ (MMS™) has significantly enhanced the analyte assignment capabilities of the HRT+ by including both positive and negative chemical ionization modes to aid in the determination of molecular formulae for a wider array of analytes. The utilization of one source for collection of complementary Electron Ionization (EI), Positive Chemical Ionization (PCI), and Electron Capture Negative Ionization (ECNI) high resolution mass spectral data has streamlined the characterization of complex samples. The *MMS,* together with ChromaTOF® brand software, provides the advantages of automated optimization, easy transition between the three ionization modes, increased sensitivity for soft ionization, improved chromatographic peak shape, and excellent retention time alignment (Figure 1).

In this application note, a mixture of EPA 8270 MegaMix and 525.3 Organochlorine pesticide (OCP) standards were analyzed and the results were used to demonstrate the benefits of *MMS* ionization and high resolution time-of-flight mass spectrometry.

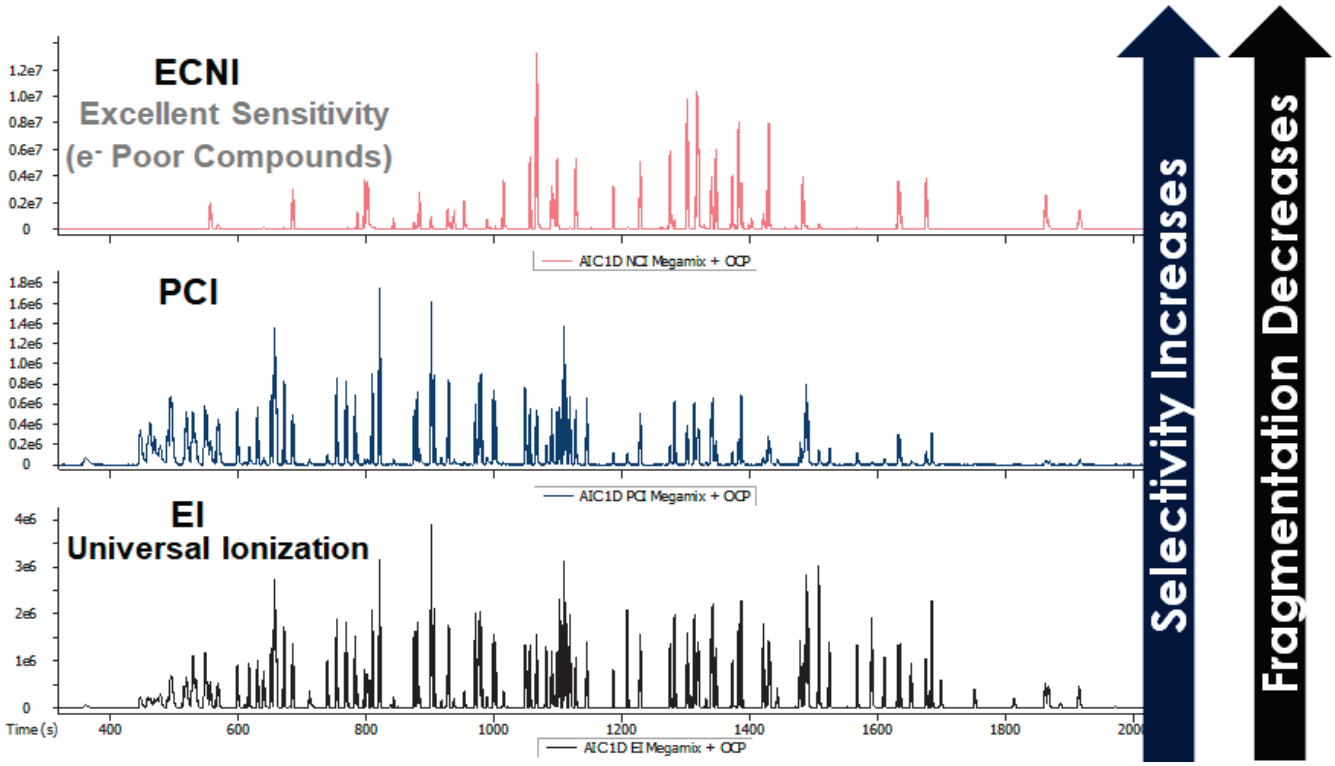


Figure 1. EI, PCI, and ECNI mass spectra for EPA Method 8270 MegaMix and 525.3 Oranochlorine Pesticide (OCP) standards.

Experimental

EPA 8270 MegaMix (Restek, Cat. No. 31686) and EPA Method 525.3 OCP Cal standards (Restek, Cat. No. 32542) were mixed and diluted with dichloromethane to a concentration of 2 ng/µL. Data for this standard mixture were acquired using an *MMS* and *Pegasus* GC-HRT$^+$ operating under the conditions shown in Table 1.

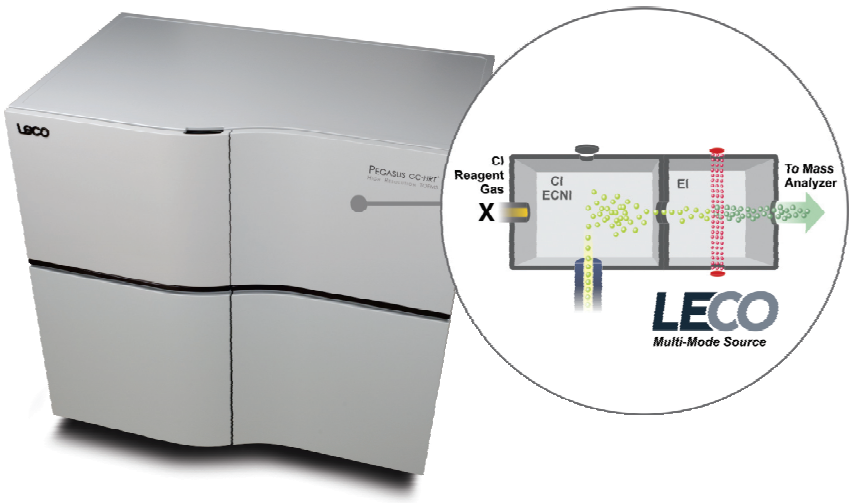
Table 1

Gas Chromatograph	**Agilent 7890B**		
Injection	1 µL Splitless, 250 °C		
Carrier Gas	He @ 1.0 mL/min, Corrected Constant Flow		
Column	Rxi -5ms, 30 m x 0.25 mm ID x 0.25 µm		
Temperature Program	40 °C (1 min) to 300 °C @ 10 °C/min; 10 min hold		
Mass Spectrometer	**LECO *Pegasus* GC-HRT$^+$**		
Ionization Mode	***EI***	***PCI***	***ECNI***
Source Temperature (° C)	250	165	165
Electron Energy (eV)	70	140	130
Emission Current (mA)	0.5	0.1	0.05
CH_4 Flow Rate (mL/min)	N/A	1.2	2.9
Acquisition Rate (Hz)	12	12	12
Mass Range (m/z)	45-1000	60-1000	30-1000

MMS Ionization Modes

1) Electron Ionization (EI):

EI is a universal, reproducible mode of ionization that produces mass spectra that correlate well with large, established databases (e.g., NIST, Wiley). Furthermore, it provides valuable fragmentation information that is used for structural characterization. The limitation of EI is that for some analytes, this mode is too energetic, and often results in absent or low intensity molecular ions, which makes unknown identification challenging. For analytes that do not exhibit a molecular ion via EI, softer *MMS* ionization modes (PCI and ECNI) can provide valuable molecular information.



2) Positive Chemical Ionization (PCI):

PCI is a soft ionization technique that provides formula information for compounds that do not contain molecular ions in their EI mass spectra. PCI occurs in three major steps: 1) Primary ion formation, 2) reagent ion formation, and 3) adduct formation (Figure 2).[11] In the first step, reagent gas is ionized to produce cations and radical cations that react further to produce reagent ions which are strong Lewis acids (Step 2). These acids then react with molecules to produce positively-charged molecular adducts (Step 3).

1) Primary ion formation:

$$CH_4 + e^{\ominus} \rightarrow CH_4^{\oplus\bullet} + CH_3^{\oplus} + CH_2^{\oplus\bullet} + CH^{\oplus} + C^{\oplus\bullet} + H_2^{\oplus\bullet} + H^{\oplus}$$

2) Reagent ion formation:

$$CH_4^{\oplus\bullet} + CH_4 \rightarrow CH_5^{\oplus} + CH_3^{\cdot}$$
$$CH_3^{\oplus} + CH_4 \rightarrow C_2H_5^{\oplus} + H_2$$
$$CH_4 + C_2H_3^{\oplus} \rightarrow C_3H_5^{\oplus} + H_2$$

3) Adduct Formation:

$$M + CH_5^{\oplus} \rightarrow [MH]^{\oplus} + CH_4$$
$$M + C_2H_5^{\oplus} \rightarrow [M+C_2H_5]^{\oplus}$$
$$M + C_3H_5^{\oplus} \rightarrow [M+C_3H_5]^{\oplus}$$
$$AH + CH_5^{\oplus} \rightarrow A^{\oplus} + CH_4 + H_2$$

Figure 2. The Major Steps of PCI: Primary Ion, Reagent Ions, and Adduct Formation.

3) Electron Capture Negative Chemical Ionization (ECNI)

ECNI is an ideal ionization mode for compounds with high electron affinity. ECNI is a soft ionization mode that uses buffer gases such as methane or argon to produce low energy electrons (Figure 3). The buffer reduces the energy of filament generated electrons (e^-) through collisions to produce lower energy, "thermal electrons" ($*e^-$).[11] The thermal electrons react with electron poor (high electron affinity) molecules to produce anions via three different mechanisms: 1) Associative resonance capture, 2) dissociative resonance capture, and 3) ion pair formation. The exact mechanism for ionization will depend on the structural and electronic characteristics of the molecules subjected to ECNI.

Formation of Thermal Electrons ($*e^-$): CH_4 (buffer gas)+ e^- → + $CH_4^{+\bullet}$ + $*e^-$ + e^-

CH_4 (buffer gas)+ e^- → + CH_3^+ + $H^\bullet$ + $*e^-$ + e^-

1) Associative Resonance Capture: $M + *e^- \rightarrow M^{-\bullet}$

2) Dissociative Resonance Capture: $M\text{-}X + *e^- \rightarrow M^- + X^\bullet$

3) Ion-Pair Formation: $M\text{-}X + *e^- \rightarrow M^+ + X^- + e^-$

Figure 3. Thermal Electron Formation and Ion Formation and Three Mechanisms of ECNI: 1) Associative Resonance Capture, 2) Dissociative Resonance Capture and 3) Ion-Pair Formation.

Results and Discussion

The examples shown in the narrative that follows represent data obtained using the *MMS* and different ionization modes for representative compounds in the Megamix and OCP standard mixture. For example, the mass spectra for 2-nitrophenol, a molecule with both an electron donating (-OH) and electron withdrawing group ($-NO_2$), are compared in Figure 4. The EI spectrum compares favorably with its NIST database equivalent (Similarity = 879/1000). The mass accuracy for its molecular ion is 0.10 ppm. The complementary PCI spectrum includes molecular adducts at m/z 140.03424 ($[MH]^+$, 0.08 ppm), 168.06578 ($[M+C_2H_5]^+$, -1.43 ppm) and 180.06546 ($[M+C_3H_5]^+$, -0.33 ppm). In addition, there is a fragment ion at m/z = 122.02362 ($[M\text{-}OH]^+$, -0.29 ppm). The isotopic fidelity, which is a comparison between the observed and theoretically calculated ion isotope cluster for the protonated molecular ion, was 958 out of a possible perfect score of 1000.

There is significantly less fragmentation, and an intense molecular anion at m/z 139.02739 (-0.74 ppm) in the ECNI spectrum for 2-nitrophenol. The isotopic fidelity for the radical anion was 990/1000. As mentioned in the experimental section, the exact mechanism of ECNI depends on the structural and electronic characteristics of compounds. For example, the molecular anion of 2-nitrophenol is formed via associative resonance capture while ionization of the pesticide lindane occurs through dissociative resonance capture to produce an $[M\text{-}Cl]^-$ fragment ion (Figure 5). The base peak in the ECNI spectrum of lindane, $[Cl_2]^-$, and a resonance-stabilized cation (not detected) are produced through ion-pair formation. The isotopic fidelity and mass accuracy for the $[M\text{-}Cl]^-$ anion in the spectrum for lindane were 987/1000 and 0.03 ppm respectively.

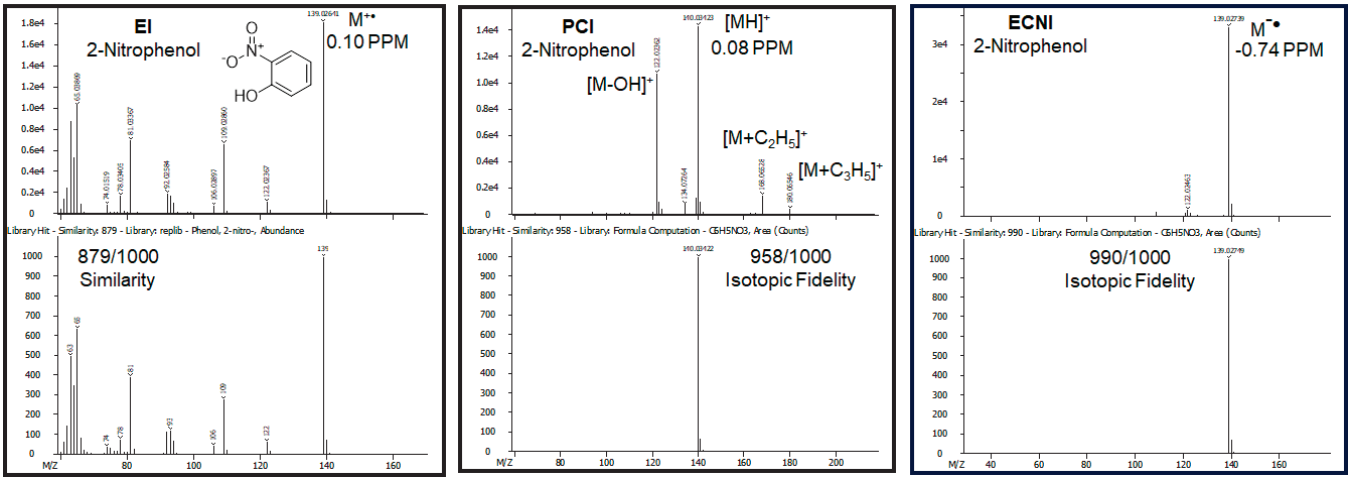


Figure 4. EI, PCI & ECNI Mass Spectral Data for 2-Nitrophenol.

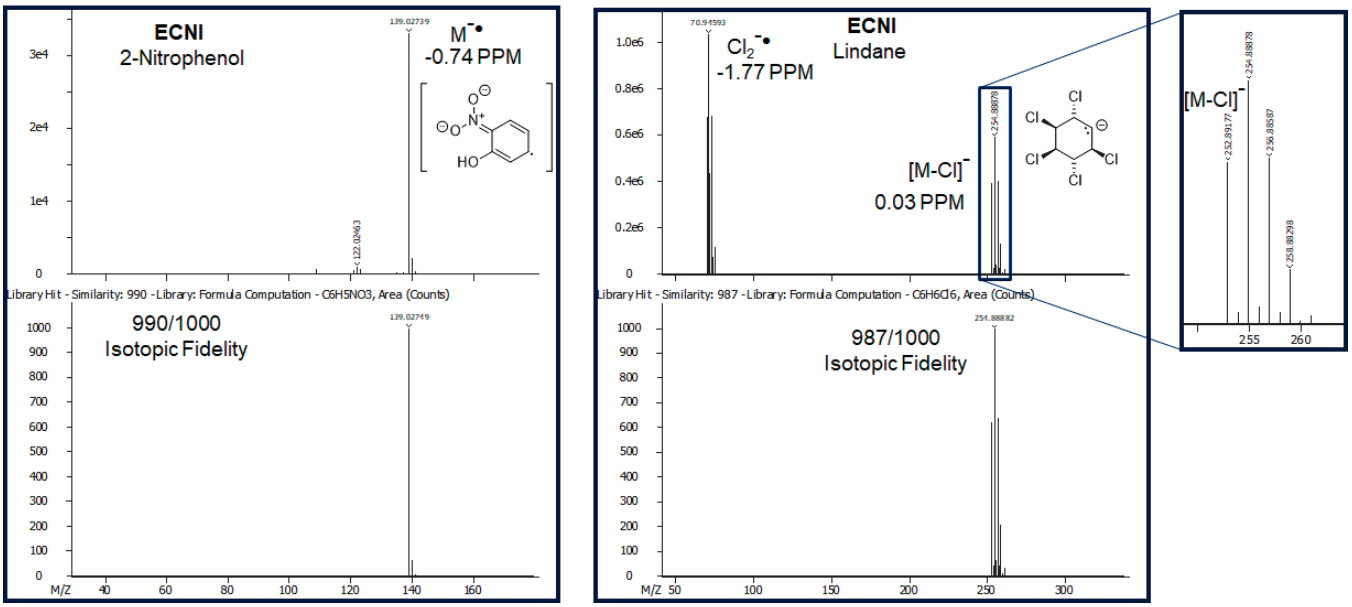


Figure 5. Comparison of ECNI Mass Spectral Data for 2-Nitrophenol & Lindane.

The EI and PCI mass spectra for the electron-rich compound 2,4-dimethylphenol are displayed in Figure 6. The EI mass spectra has a NIST library similarity score of 941/1000 and a molecular ion mass accuracy of 0.28 ppm. The PCI spectrum exhibits excellent isotopic fidelity (982/1000) with a mass accuracy of -0.46 ppm for the protonated molecular ion at m/z 123.06039. The mass accuracy values for the corresponding $[M+C_2H_5]^+$and $[M+C_3H_5]^+$ adducts were -0.14 and -0.19 ppm respectively. Electron rich 2,4-dimethylphenol does not ionize under ECNI conditions.

There are electron deficient compounds in the standard mixture, such as 2-methyl-1,3-dinitrobenzene, that ionize in all three modes (Figure 7). The molecular ion is absent in the EI spectrum of 2-methyl-1,3-dinitrobenzene; but confident characterization can be accomplished through analysis of the high resolution, accurate mass fragment ions, and spectral library searches (864/1000). Fortunately, there is a strong protonated molecular ion at m/z 183.04002 (-0.07 ppm, Isotopic Fidelity 984/1000) in the complementary PCI spectrum. This spectrum also contains additional adducts at m/z 211.07105 ($[M+C_2H_5]^+$, -1.34 ppm) and m/z 223.07135 ($[M+C_3H_5]^+$, 0.08 ppm). The ECNI spectrum has virtually no fragmentation, and an intense molecular anion at m/z 182.03315 (-0.85 ppm, Isotopic Fidelity Score 982/1000). This anion is more intense (> 100X) than any of the ions in the EI or PCI spectra.

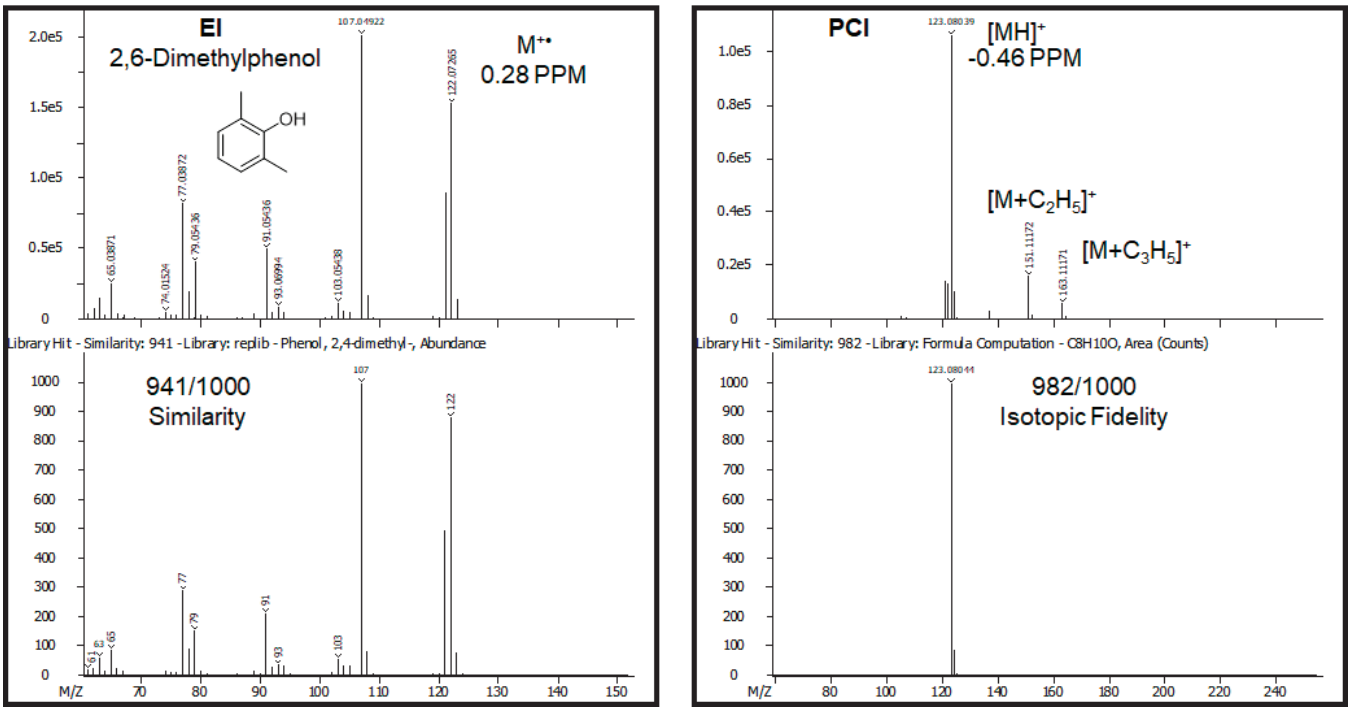


Figure 6. EI and PCI Mass Spectral Data for 2,4-Dimethylphenol.

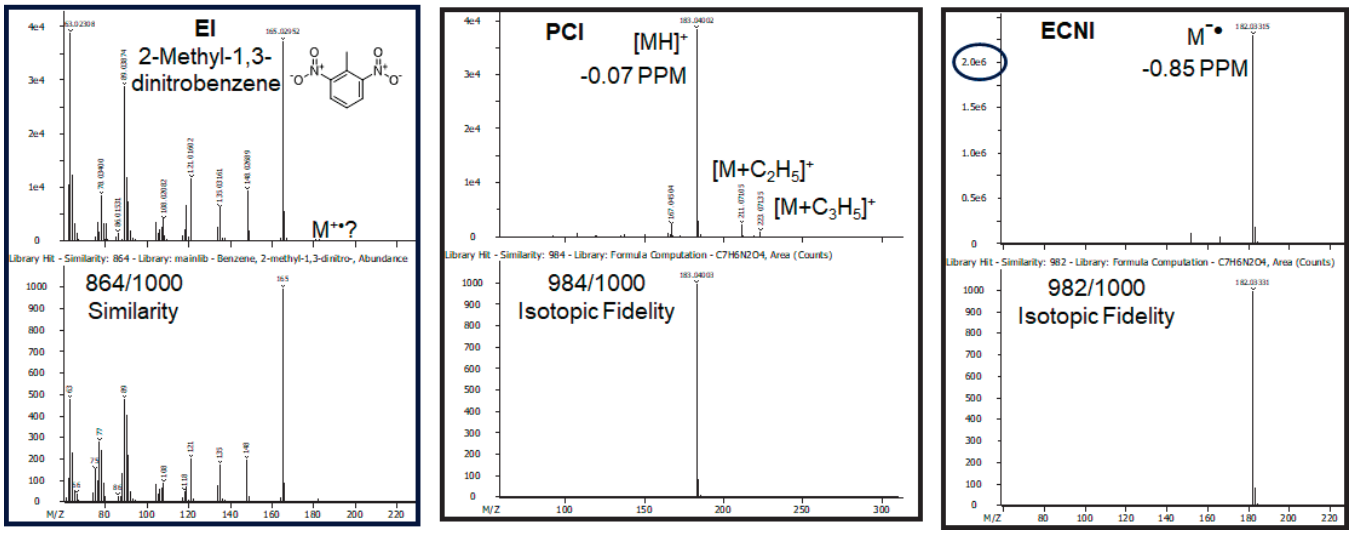


Figure 7. EI, PCI and ECNI Mass Spectral Data for 2-Methyl-1,3-Dinitrobenzene.

The combination of EI and ECNI analysis is exceptionally suited for the analysis of polyhalogenated organic compounds. For example, this approach is ideal for the analysis of polychlorinated organic compounds. First, EI data is used to identify these compounds through a combination of spectral similarity searches and formula determinations using the high-resolution accurate mass ions. Second, ECNI spectra with enhanced selectivity and sensitivity is produced for these electron deficient compounds resulting in high-resolution spectra with intense anions that can be used to support the EI results. The implementation of this methodology is illustrated for endosulfan sulfate, chlordane and α-endosulfan (Figures 8-10). The EI spectra for endosulfan sulfate, chlordane, and a-endosulfan exhibit excellent spectral similarity values (929, 945, and 909/1000). The molecular ion mass accuracies for endosulfan sulfate and chlordane were -0.33 and -0.10 ppm. There was no molecular ion present in the EI mass spectrum for α-endosulfan. Careful inspection of spectra demonstrates a decrease in fragmentation and increase in sensitivity for each of these pesticides when transitioning from EI to ECNI spectra. The corresponding mass accuracy values for the ECNI generated molecular anions in the spectra for endosulfan sulfate, chlordane, and α–endosulfan were 0.28, -0.62, and -0.97 ppm with isotopic fidelities of 996, 992, and 962/1000.

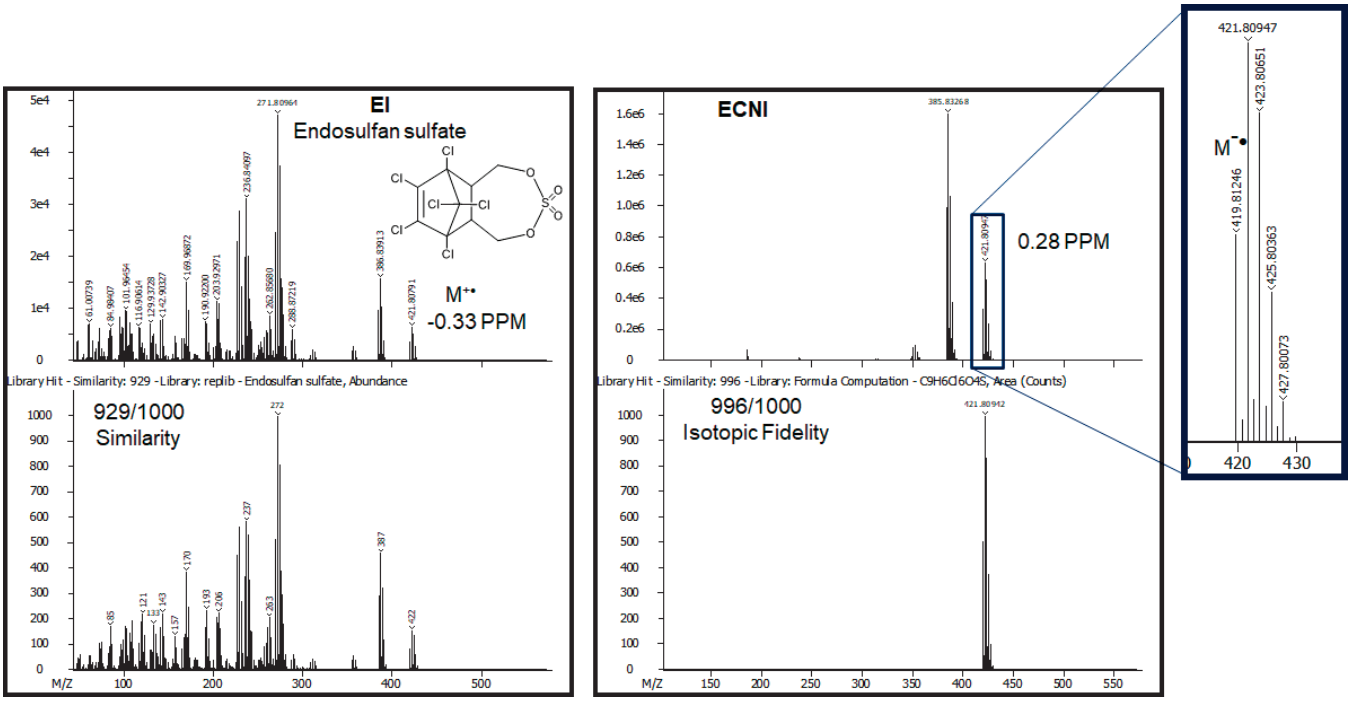


Figure 8. EI and ECNI Mass Spectral Data for Endosulfan Sulfate.

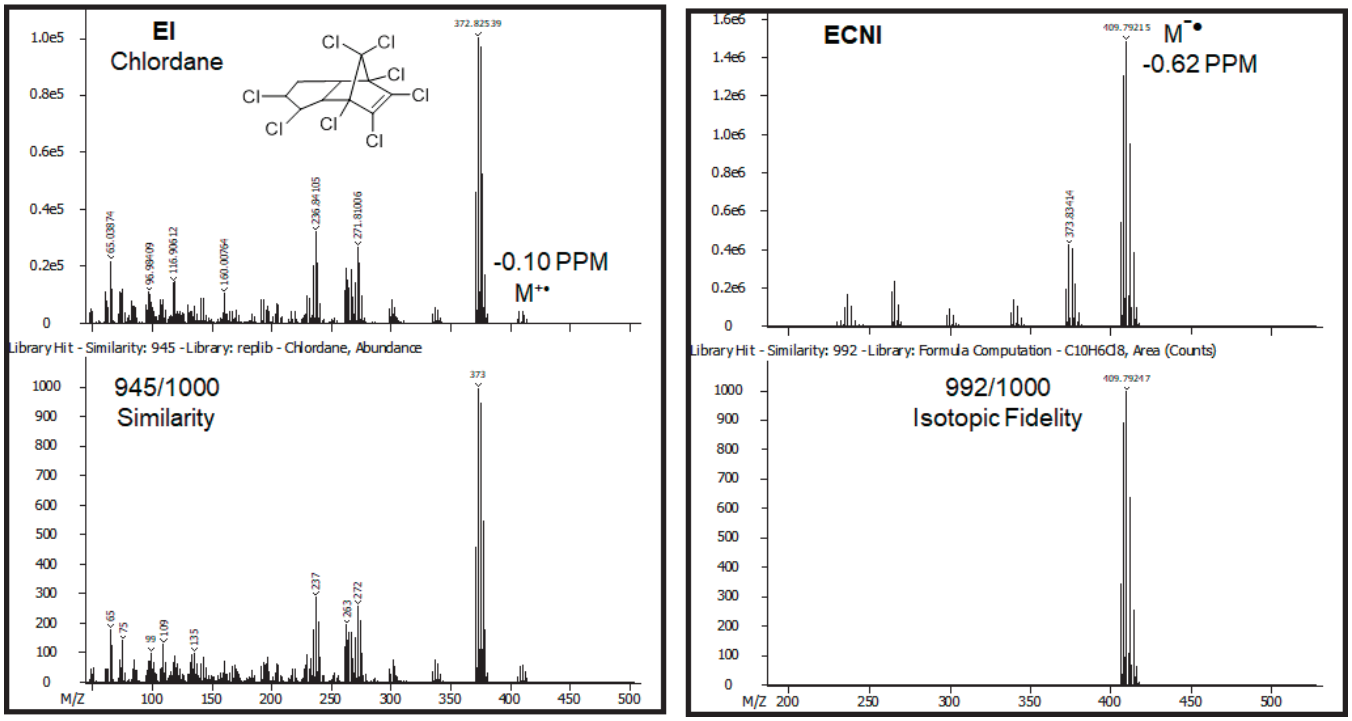


Figure 9. EI and ECNI Mass Spectral Data for Chlordane.

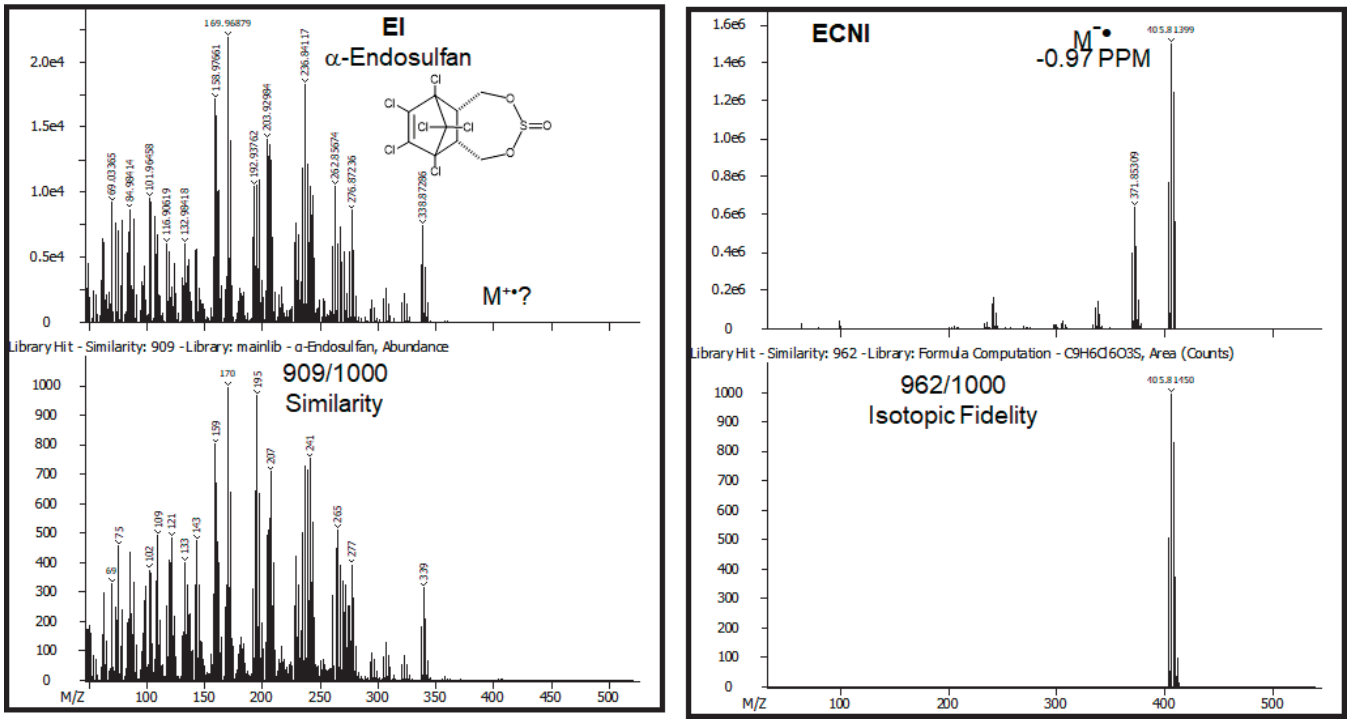


Figure 10. EI and ECNI Mass Spectral Data for α-Endosulfan.

Conclusion

The *Pegasus* GC-HRT$^+$ with *MMS* is a unique and powerful problem-solving tool for the analysis of complex samples. The MMS source provides exceptional mass spectral data with excellent spectral similarity scores, accurate mass formulae (<1 ppm) and high isotopic fidelity values for molecular, adduct, and fragment ion clusters. EI data were utilized to identify compounds through spectral similarity and formula determinations using high-resolution accurate mass ions. PCI and ECNI spectra facilitated compound characterization by providing molecular information, in addition to improved selectivity for compounds depending on their structural and electronic characteristics.

References

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