Unambiguous Pesticide Identification in Cigarette Tobacco

LECO Corporation; Saint Joseph, Michigan USA

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1. Introduction

This note highlights the capability of high resolution time-of-flight (HRT) mass spectrometry to confidently identify analytes in a complex matrix, while maintaining the ability to screen for other components such as additives and natural ingredients in the tobacco. This was accomplished utilizing ChromaTOF-HRT® brand software with High Resolution Deconvolution™ (HRD™) which leverages the Pegasus® GC-HRT's industry-leading resolution and mass accuracy. A wide variety of pesticides are used extensively on tobacco crops (i.e., 37 pesticides approved by the USEPA are used regularly on tobacco crops). Tobacco is a complex matrix that can be challenging analytically, often requiring rigorous sample preparation before instrumental analysis which can be prone to false positives and false negatives. High resolution mass spectrometry (HRMS) is necessary to separate ions that have the same nominal mass from one another to ensure that only diagnostic (or characteristic) ions are assigned to any given analyte, thus minimizing the risk of false positives and false negatives. These accurate mass measurements are a requisite for accurate chemical formula determination and for detecting analytes in complex matrices so as to avoid isobaric interferences or to separate coeluting analytes. The current objective was to confidently identify pesticide residues in cigarette tobacco with limited sample cleanup. QuEChERS sample preparation was followed by gas chromatography high resolution time-of-flight mass spectrometry on a LECO *Pegasus* GC-HRT.
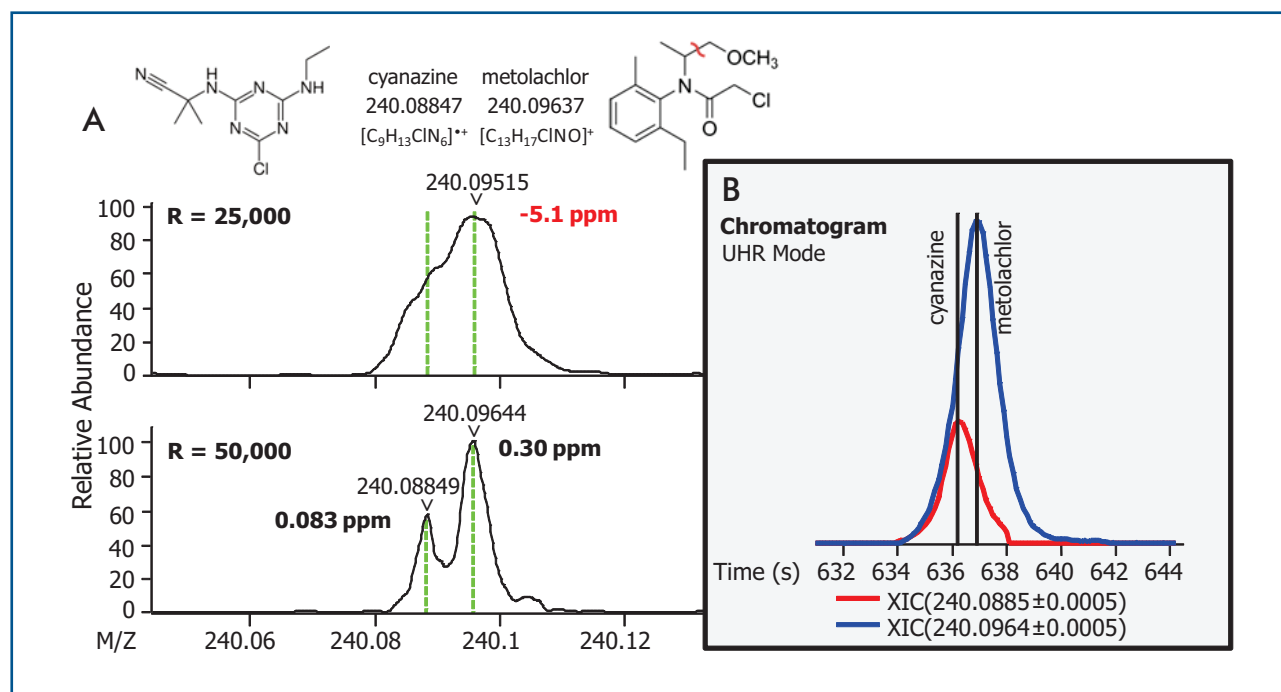

Figure 1. *The pesticides cyanazine and metolachlor have isobarically convoluted masses at a 2:1 ratio that could only be resolved with a mass resolution greater than 40,000. The profile mass spectra collected at a resolution of 25,000 (top A) show incorrect mass measurements, while correct measurements were obtained (bottom A) collecting in 50,000 resolution mode. Inset B shows the ability of HRD to effectively deconvolve and identify these analytes.*

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2. Experimental

Samples: Cigarette tobacco was removed from a national brand of cigarettes and extracted using QuEChERs sample preparation as described elsewhere.[1] The tobacco extract was split into two fractions: one for the discovery of incurred pesticides, and the other was fortified with a mixture of pesticides.

Data Analysis: Data were collected and processed with LECO's *ChromaTOF-HRT* brand software. Analyte peak finding consisted of high resolution deconvolution (HRD) and library database searching with accurate mass confirmation.

Gas Chromatograph	Agilent 7890 with 7693 Autosampler
Injection	1μL, Splitless @ 250°C
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column	Rxi-5 Sil MS, 30 m x 0.25 mm i.d. x 0.25 μm (Restek, Bellefonte, PA, USA)
Temperature Program	60 °C (1 min), to 160°C @ 25°C/min, to 190°C @ 2.5°C/min, to 205°C @ 3.0°C/min, to 220°C @ 5.0°C/min, to 285°C @ 17°C/min (10 min)
Mass Spectrometer	**LECO *Pegasus* GC-HRT**
Transfer Line	275°C
Acquisition Mode	High Resolution, R = 25,000 (FWHM); Ultra High Resolution, R = 50,000 (FWHM)
Ion Source Temperature	250°C
Ionization Mode	EI
Mass Range (m/z)	45-550
Acquisition Rate	8 spectra/s

3. Results and Discussion

Figure 1 shows an example of when it is necessary to separate coeluting analytes, whereby the molecular ion of cyanazine and a fragment ion of metolachlor differ in exact mass by ~7.95 mDa. The mass accuracy of -5.1 ppm at 25,000 resolution (Figure 1A) was an indicator that an isobaric interference may have been present. Typically for LECO's *Pegasus* GC-HRT, mass accuracy values are within ±1.0 ppm, so any deviation from this norm signifies a potential concern to the user, and a value more than 2 ppm suggests the need for further investigation. At 50,000 resolution the molecular ion of cyanazine and the fragment ion of metolachlor are clearly resolved with mass accuracies of 0.083 ppm and 0.30 ppm, respectively. Additionally, these ions occur at a 2:1 ratio in the sample which increases the resolution requirement. A resolution less than 40,000 will result in a false negative for the molecular ion of cyanazine because it is masked by a fragment ion of metolachlor, making it impossible to perform accurate quantitation for the molecular ion of cyanazine.

Figure 2 demonstrates the advantage of HRMS for the isolation of ions from matrix interferences. The base peak of dichlorvos (m/z = 109.0049), which is the primary ion recommended for its quantification, is only distinguishable from the tobacco matrix with a resolution greater than 10,000 FWHM.
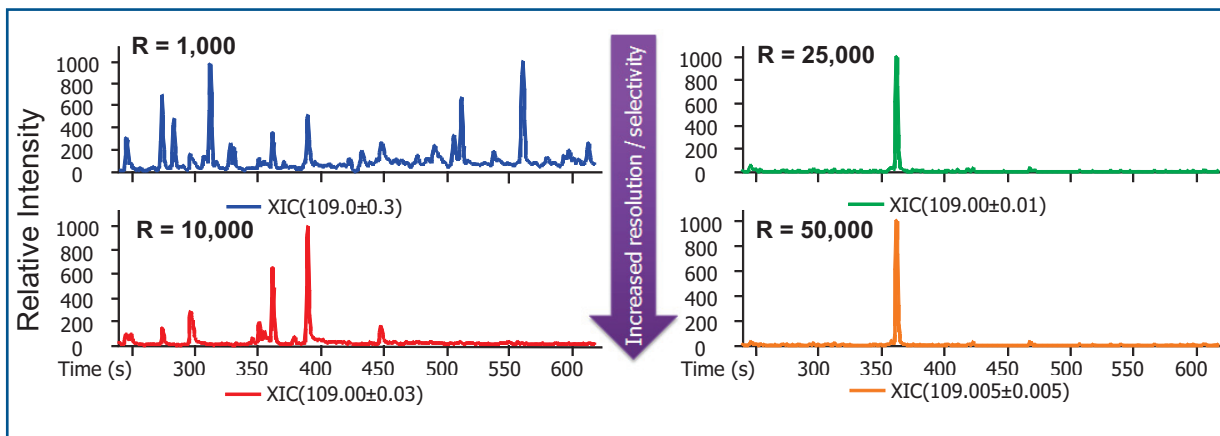

Figure 2. A mass resolution greater than 10,000 was required to unequivocally extract the base peak of dichlorvos (m/z = 109.0049) from the tobacco matrix.
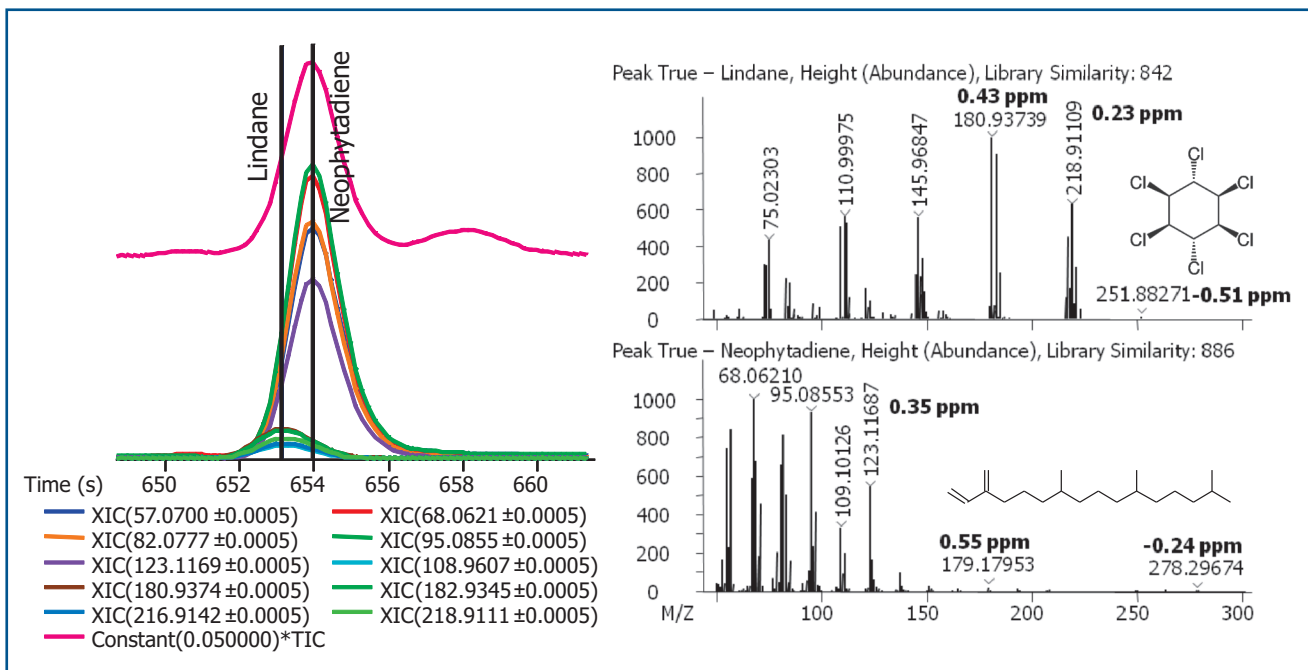
Figure 3. (Left) The extracted ion chromatograms (Xic) for the most abundant masses of lindane (10 ppb) and neophytadiene, a prevalent component in the tobacco matrix, were identified as a result of HRD. Peak True (deconvoluted) mass spectra for lindane and neophytadiene have good library matches strengthened by accurate mass measurements for fragment and molecular ions.

Figure 3 highlights the benefits of LECO's *ChromaTOF-HRT* brand software with HRD, which is seamlessly integrated within the peak finding algorithm. In this example, the pesticide lindane is masked ostensibly by a matrix interference, neophytadiene, when viewed as a total ion chromatogram (TIC); however, the HRD software was able to differentiate the ions corresponding to each compound, resulting in library matches greater than 800 for each.

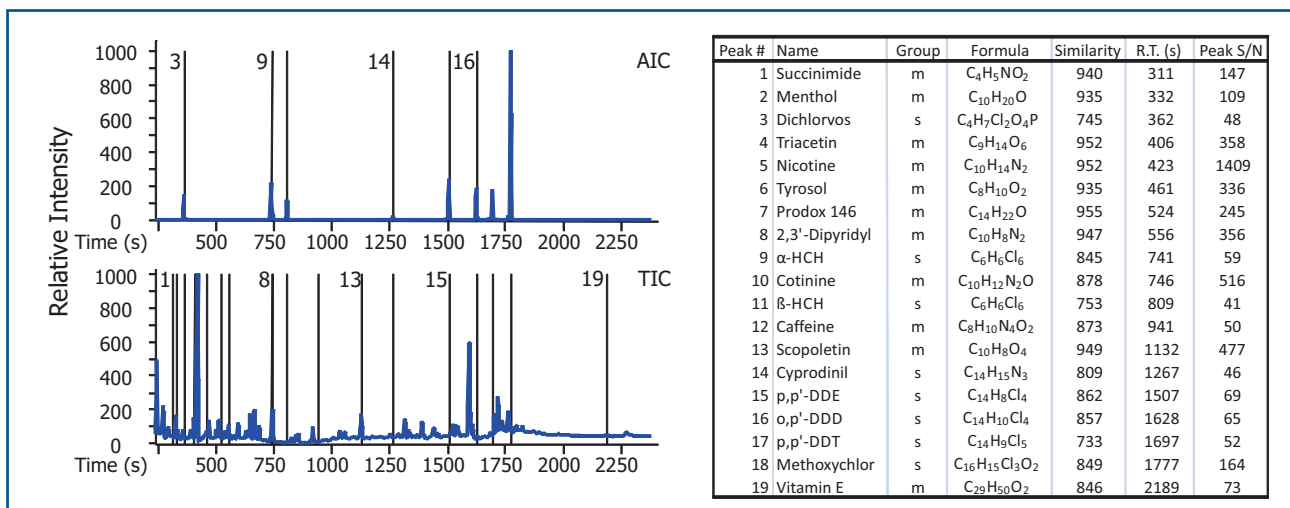| Peak #	Name	Group	Formula	Similarity	R.T. (s)	Peak S/N
1	Succinimide	m	$C_4H_5NO_2$	940	311	147
2	Menthol	m	$C_{10}H_{20}O$	935	332	109
3	Dichlorvos	s	$C_4H_7Cl_2O_4P$	745	362	48
4	Triacetin	m	$C_9H_{14}O_6$	952	406	358
5	Nicotine	m	$C_{10}H_{14}N_2$	952	423	1409
6	Tyrosol	m	$C_8H_{10}O_2$	935	461	336
7	Prodox 146	m	$C_{14}H_{22}O$	955	524	245
8	2,3'-Dipyridyl	m	$C_{10}H_8N_2$	947	556	356
9	α-HCH	s	$C_6H_6Cl_6$	845	741	59
10	Cotinine	m	$C_{10}H_{12}N_2O$	878	746	516
11	ß-HCH	s	$C_6H_6Cl_6$	753	809	41
12	Caffeine	m	$C_8H_{10}N_4O_2$	873	941	50
13	Scopoletin	m	$C_{10}H_8O_4$	949	1132	477
14	Cyprodinil	s	$C_{14}H_{15}N_3$	809	1267	46
15	p,p'-DDE	s	$C_{14}H_8Cl_4$	862	1507	69
16	o,p'-DDD	s	$C_{14}H_{10}Cl_4$	857	1628	65
17	p,p'-DDT	s	$C_{14}H_9Cl_5$	733	1697	52
18	Methoxychlor	s	$C_{16}H_{15}Cl_3O_2$	849	1777	164
19	Vitamin E	m	$C_{29}H_{50}O_2$	846	2189	73

Figure 4. TIC of the fortified tobacco extract highlighting a number of spiked pesticides (s) and incurred components in a tobacco matrix (m). HRMS by time-of-flight provides the ability to confidently detect and identify non-target compounds while maintaining the selectivity to perform unambiguous identification of your target analytes. The analytical ion chromatogram (AIC) shows the sum of the deconvoluted masses for each pesticide listed in the table.

What else is in your sample? The AIC in Figure 4 illustrates a chromatogram similar to that observed when collecting data in selected ion-monitoring mode on a scanning instrument for the pesticides listed in the table. The TIC represents the extensive information available when collecting data comprehensively using time-of-flight. The table is a brief summary of the matrix related compounds that were identifiable simultaneously with pesticides all in one sample acquisition.

4. Conclusion

This application note demonstrates a few of the many benefits of HRT mass spectrometry for the analysis of pesticides in complex matrices like cigarette tobacco. With industry-leading mass accuracy and resolution, the *Pegasus* GC-HRT provides increased selectivity, enabling the isolation of analytes from the matrix and helping to win the fight against coelution. The user can confidently identify compounds in this challenging matrix, while maintaining the ability to screen for other components such as additives and natural ingredients in tobacco.

5. Reference

[1]Misselwitz, M.; Cochran, J.; Kowalski, J. Evaluation of dispersive and cartridge solid phase extraction (SPE) cleanup for multiresidue pesticides in QuEChERS extracts of finished tobacco using GCxGC-TOFMS. http://www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff_FFAN1823-UNV.