Ion Ratio as Quality Assurance for Pesticide Analysis by Gas Chromatography—Time-of-Flight Mass Spectrometry (GC-TOFMS)

Introduction

Ion Ratio is a ChromaTOF® software feature that was originally developed for clients who prior to their acquisition of a LECO Pegasus Gas Chromatograph (GC)—Time-of-Flight Mass Spectrometer (TOFMS), were measuring polychlorinated biphenyls (PCBs) by GC-Selected Ion Recording (SIR)—high-resolution mass spectrometry (HRMS). With SIR, where a full mass spectrum is not available, to ensure that it was a PCB they were determining and not an interference, they compared the ratios of two molecular ions for each congener (Figure 1). In nature, ^{35}Cl and ^{37}Cl exist in a 76% and 24% proportion, respectively. Exact ion ratios for different chlorination levels can be calculated, and if SIR determined ratios were outside a percentage range for the expected ratios (either theoretical or from analyzed standards), then the particular compound was not a PCB. Conversely, if the measured and expected ratios matched within a certain error (and the GC retention time was correct), then the compound was a PCB. This method is also used in chlorinated dioxin and furan analysis with SIR HRMS. Even though these clients now had the qualitative power of a full mass spectrum from a time-of-flight mass spectrometer, they desired Ion Ratio to supplement PCB verification.

Ion Ratio can be used for pesticide analysis as a Quality Assurance (QA) tool to supplement the full mass spectrum that is always obtained when doing TOFMS. Most importantly, Ion Ratio may illuminate quantification mass bias from interferences in the case where summed ions are used for quantification. This application note demonstrates Ion Ratio for a group of pesticides analyzed in a spiked spinach extract.

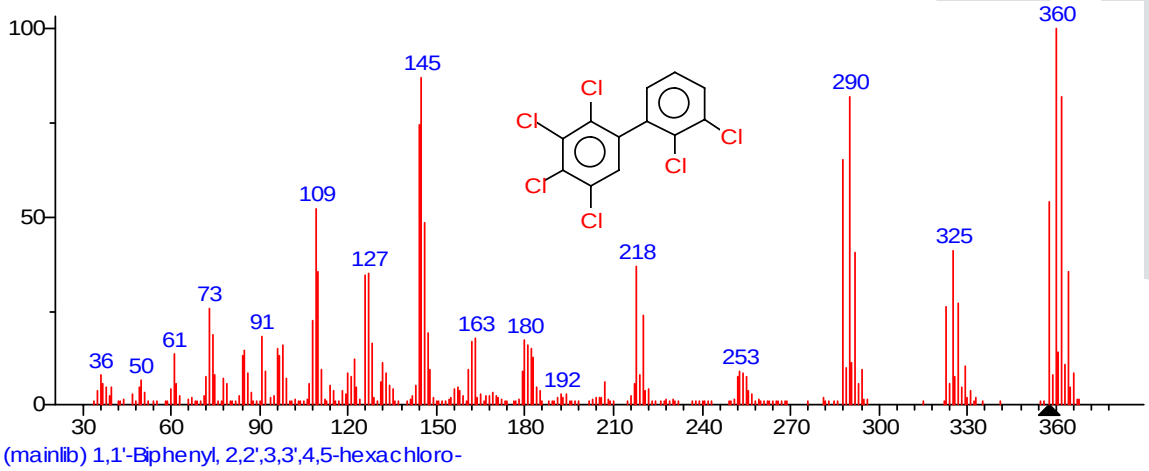


Figure 1. *Mass spectrum of hexachlorobiphenyl. The 360 and 362 ions would be used for selected ion recording—high-resolution mass spectrometry. Their proportion, as calculated from ^{35}Cl and ^{37}Cl abundances in nature, is approximately 1.25 (360/362).*

Experimental Conditions

Gas Chromatography: Agilent 6890 GC

Column:	20 m x 0.18 mm x 0.14 µm CLPII (Restek)
Carrier:	Helium at 1 mL/min, constant flow
Injection:	1 µL splitless at 250°C, 60 sec. valve
Oven Program:	40°C (1 min), 40°/min to 120°, 20°/min to 320°
Total Run Time:	13 min

Mass Spectrometry: LECO Pegasus III TOFMS

Ionization:	Electron ionization at 70eV
Source Temperature:	225°C
Stored Mass Range:	45 to 550 u
Acquisition rate:	20 spectra/sec.

Data Processing

LECO ChromaTOF software with automatic Peak Find and Deconvolution

Extraction and Analysis of Spinach

The Florida-Modified—California Department of Food and Agriculture multiresidue method was used to prepare an extract from frozen spinach purchased at a local grocery store. The spinach extract was spiked with pesticides prior to analysis by GC-TOFMS.

Calibration and Ion Ratio for Pesticide Analysis

With the full mass acquisition that is always available in TOFMS, the choice for identifying a compound in a sample as a pesticide, and then quantifying that pesticide is usually a three step process.

1. Does the compound meet the pesticide Reference Spectrum match factor?
2. Does the compound fall within a certain retention time window?
3. Does the compound meet the S/N or area threshold set by the user?

However, there is a possibility that a mass spectrum of an identified pesticide can meet the Reference Spectrum criterion, but still show bias (due to an interfering compound) on a mass selected for quantification. In this case, Ion Ratio can be an excellent QA feature for flagging the quantification bias.

Ion Ratio is set up in a Calibration Table (Figure 2). After analysis of pesticide standards, Ion Masses are entered in the Calibration Table and their ratios are calculated from a higher level standard. The user can define an Ion Ratio Tolerance (%) that will govern whether the analyzed ratio of a pesticide in a sample will be flagged as "Passed" or "Failed" (Figure 3). An Ion Ratio Result marked "Passed" supplements the Reference Spectrum match, which is the first step in identifying a peak in the proper retention time window as a particular pesticide. "Passed" also assures quantitative accuracy by indicating that no bias exists on masses chosen for quantification.

Calibration Table - "PDP Calib Ion Ratio Ltd"

Analyte	Name	Absolute R.T. (s)	Ion Ratio Masses	Expected Ion Ratio	Ion Ratio Tolerance (%)
1*	Trifluralin	426.394	264/306	1.22	30.0
2	Pentachloronitrobenzene	465.594		Not Defined	30.0
3	Chlorothalonil	503.394	264/266	0.770	30.0
4	DCPA	534.794	299/301	0.800	30.0
5	Thiabendazole	570.01	174/201	1.23	30.0
6	4,4'-DDE	576.85	316/318	0.786	30.0
7	Azinphos methyl	703.144	132/160	1.04	30.0

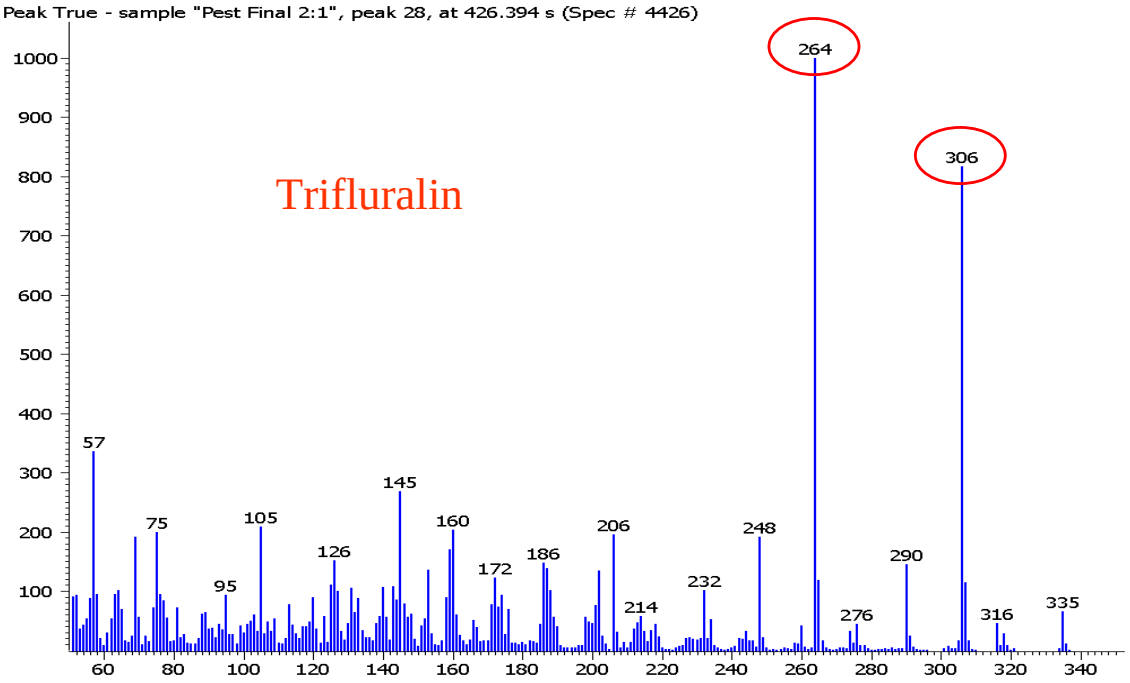


Figure 2. *Ion Ratio for a group of pesticide standards in a Calibration Table. The Trifluralin mass spectrum demonstrates how two substantial ions (264 and 306) have been chosen as the Ion Masses with an Expected Ion Ratio of 1.22.*

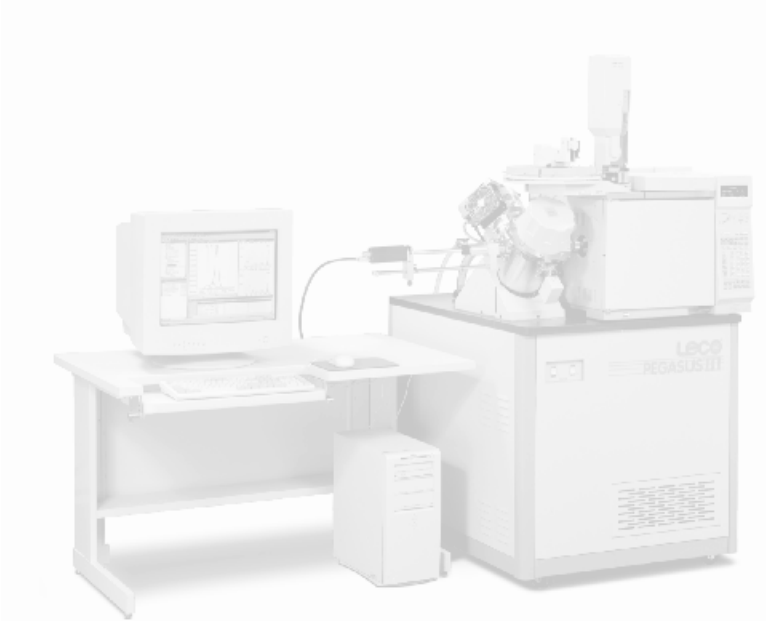
Peak Table - "Spinach 0.5:1"

Peak #	Quantification	R.T. (s)	Ion Ratio Masses	Calculated Ion Ratio	Ion Ratio Result	Quant Masses	Concentration
65*	Trifluralin	426.237	264/306	1.1973	Passed	264+306	0.55
78	Pentachloronitrobenzene	465.487	Not Defined	0.0000	Not Checked	237+249+295	2.00
91	Chlorothalonil	503.287	264/266	0.76961	Passed	264+266+268	0.41
105	DCPA	534.637	299/301	0.77972	Passed	299+301+332	0.53
119	Thiabendazole	571.537	174/201	1.2431	Passed	174+201	1.08
120	4,4'-DDE	576.587	316/318	0.78852	Passed	246+248+318	0.56
142	Azinphos methyl	703.087	132/160	1.0914	Passed	77+132+160	0.83

Figure 3. *Peak Table for a spinach extract showing results of a check of Ion Ratios for the spiked pesticides. Note that the Quant Masses and the Ion Masses overlap in most cases. If Ion Ratio Result were "Failed" instead of "Passed", this would indicate a possible bias in quantification. Note that the Expected Ion Ratio could also be displayed in the Peak Table.*

Conclusions

Ion Ratio can be used to supplement the confidence of a full mass spectrum match for a pesticide in food analysis, while indicating to the analyst that no quantification mass bias exists. It will also highlight those unusual cases where a good Reference Spectrum match was achieved, but the Ion Ratio was out of tolerance, which indicates a bias on the quantification mass or masses that could lead to the reporting of an erroneously high concentration for a pesticide.



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