Application Note

Instrument: Pegasus® BT 4D

Quantitation and Non-Target Detection of Pesticides in Spinach Extract with *Pegasus* BT 4D

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Key Words: Pesticides, GCxGC, Quantitation, Non-Target Detection

Introduction

Matrix deleteriously affecting quantitation accuracy of pesticides in food commodities has been well documented and understood. While significant improvements have been made in sample extraction strategies, cleanup of complex matrices continues to be an issue, especially as limits of detection (LOD) are decreased by various regulatory entities. The increase in separation efficiency afforded by GCxGC allows for the separation of target analytes from matrix interferences. Combined with the sensitivity available with LECO's *Pegasus* BT 4D, the ability to achieve required limits of detection, while minimizing matrix interferences to allow successful quantitation and effective identification of non-targeted pesticides, has been demonstrated. Figure 1 below highlights the strengths of GCxGC's ability to effectively separate the pesticides from the interfering heavy matrix often associated with vegetable commodities such as spinach.

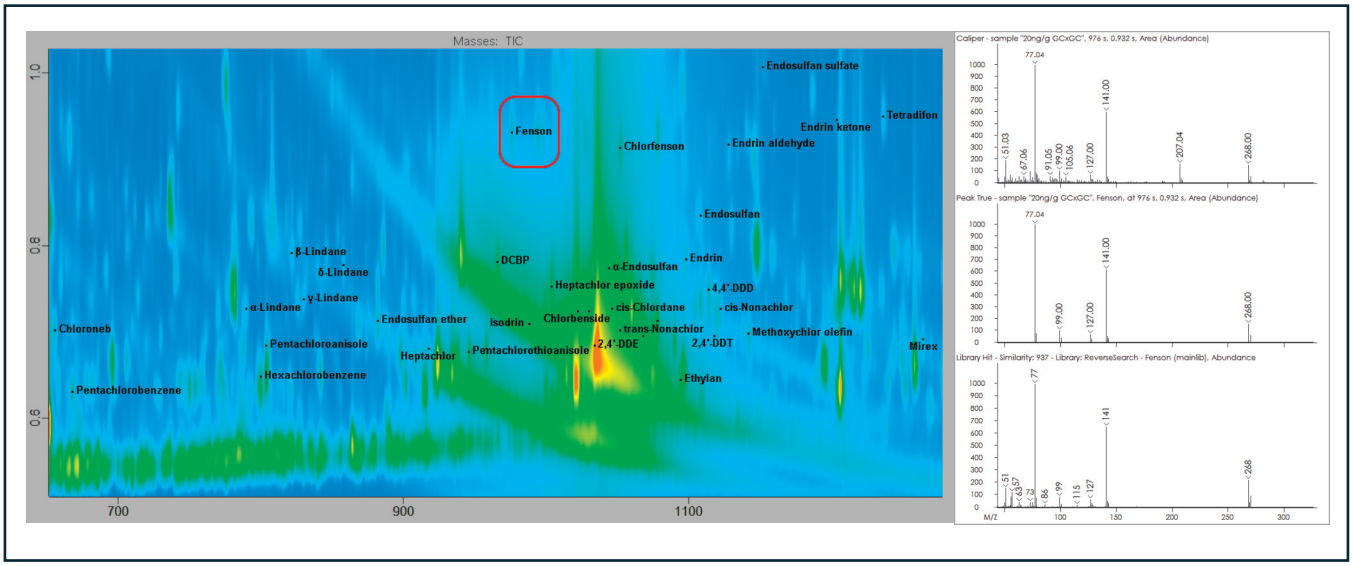

Figure 1. Section of Contour Plot of the spinach QuEChERS extract with dSPE cleanup (left) spiked with pesticides at 20 ng/g. The improvement in chromatographic separation with GCxGC significantly improves both peak detection and quantification. In this example, the second dimension of separation effectively moved the pesticide Fenson away from large matrix interferences.

Experimental

Bagged spinach was purchased from a local grocery chain. A bulk QuEChERS extract of the spinach was created following the kit instructions (www.restek.com/pdfs/805-01-001.pdf). From the final extract, a small aliquot was set aside and the remainder used to create a series of matrix matched quantitation standards. The standards were spiked at various levels with a chlorinated pesticide mix. The chlorinated mix was chosen because it is unlikely that any of the pesticides in this mix would already be present in the spinach and thus bias the quantitation results. Data for both the spiked standards and unadulterated extract were collected using conditions described in Table 1, and were processed in ChromaTOF® brand software using both Target Analyte Find (TAF) for quantitative purposes and NonTarget Deconvolution® (NTD®) peak find mode to search for other, incurred pesticides. Target peak detection, identification, and quantitation curve linearity limits for each analyte were determined following SANTE/11813/2017 guidelines for unit mass resolution TOFMS (http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727). Figure 2 shows a table from SANTE/11813/2017 where these criteria are summarized.

Table1. *Pegasus* BT 4D GCxGC-TOFMS Conditions

Mass Spectrometer	**LECO *Pegasus* BT 4D**
Ion Source Temperature	250 °C
Mass Range	45-570 m/z
Acquisition Rate	280 spectra/s (GCxGC) 8 spectra/s (GC)
Gas Chromatograph	**LECO GCxGC Quad Jet Thermal Modulator & 7693 Autosampler**
Injection	1μL Splitless @ 250 °C
Carrier Gas	He @ 1.4 mL/min, Corrected Constant Flow
Column One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 μm coating (Restek, Bellefonte, PA, USA)
Column Two	Rtx-200, 1 m x 0.25 mm x 0.25 μm coating (Restek, Bellefonte, PA, USA)
Temperature Program	1 min at 75 °C, ramped 10.2 °C/min to 320 °C, held 8 min Secondary oven maintained +5 °C relative to primary oven
Modulation	2 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	330 °C

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		Minimum number of ions	Other
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3 [d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm [a, b, c]	S/N ≥ 3 [d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

Figure 2. Table 4 of SANTE /11813/2017 describing peak identification requirements. The highlighted sections apply to Pegasus BT and BT 4D data.

Results and Discussion

Comparisons of GC and GCxGC quantitation curves show significant improvement in overall linearity and LODs with GCxGC. Examples are highlighted in Table 2, and Figures 3 and 4. These improvements are entirely due to the cryo focusing effects of thermal modulation and separation of the target analytes from the abundant spinach matrix via the second dimension of chromatographic separation.

Table 2. Comparison of GC and GCxGC quantitation results for selected pesticides. A valid quantitation curve for chlorbenside below 20 ng/g was not possible in GC due to matrix interference which was chromatographically resolved with GCxGC as shown in Figure 4.

Analyte	GC LODng/g	GC Correlation Coefficient	GCxGC LODng/g	GCxGC Correlation Coefficient
Chloroneb	5.0	0.99954	0.5	0.99977
Pentachlorobenzene	0.2	0.99901	0.1	0.99997
Pentachloroanisole	0.2	0.99915	0.1	0.99966
Heptachlor	1.0	0.99813	0.5	0.99972
Aldrin	1.0	0.99920	0.2	0.99985
Heptachlor epoxide	1.0	0.99913	0.5	0.99982
Chlorbenside	Quant Not Possible - Interference		0.5	0.99956
Dieldrin	5.0	0.99870	1.0	0.99560
Tetradifon	5.0	0.99902	0.5	0.99997
Mirex	1.0	0.99877	0.1	0.99992

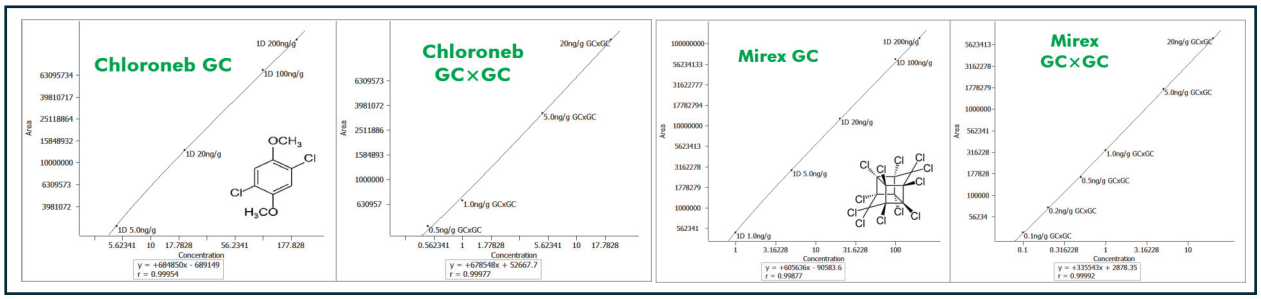

Figure 3. Example GC and GCxGC quantitation curves. The axes are scaled logarithmically to better show the bottom end of the curves.

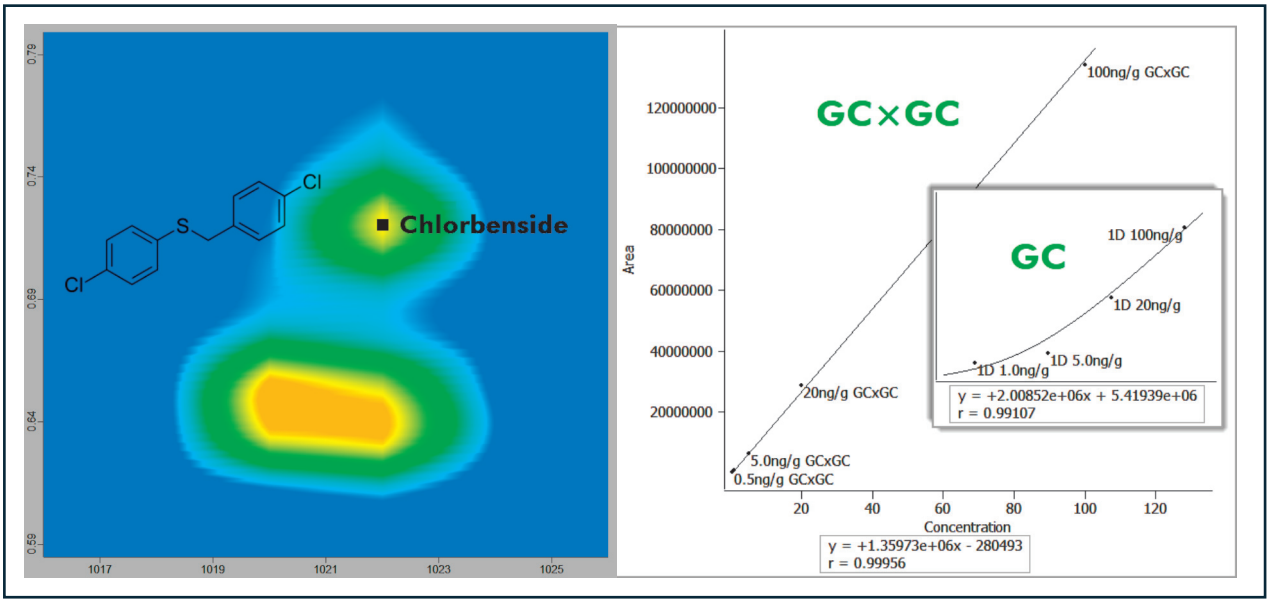

Figure 4. GCxGC resolution of Chlorbenside from the matrix interference. The GCxGC separation allows for a linear and sensitive quantitation curve. In the GC separation, the coeluting matrix completely obscures the pesticide below 20 ng/g making consistent, accurate integration impossible.

Comprehensive *NonTarget Deconvolution (NTD)* Peak Find was applied to the blank matrix extract to search for incurred pesticides and contaminants. In both the GC and GCxGC data files several pesticides as well as a likely plasticizer (possibly from the product packaging) were found (see Figure 5).

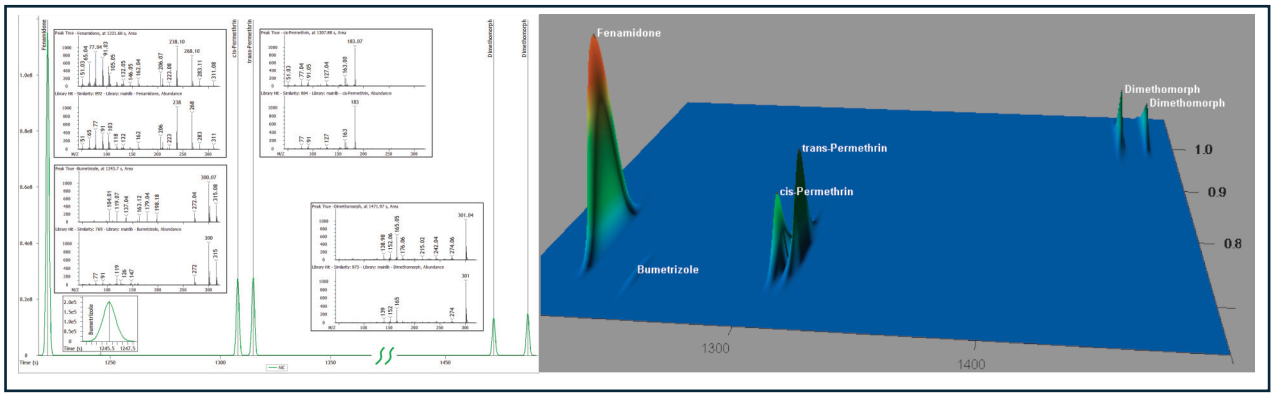

Figure 5. Initially identified incurred pesticides and plasticizer (Bumetrizole) shown in both GC Chromatograms and GCxGC Surface Plot.

An additional pesticide, Chlorantraniliprole, was found in the GCxGC data that was not originally identified in the GC data file. This compound was initially missed by GC due to a nearly perfect coelution with a large matrix peak. In Figure 6 the deconvoluted spectrum (Peak True) obtained from GC analysis shows a combination of Chlorantraniliprole and the interference successfully deconvoluted from the ubiquitous column bleed and other compounds (Caliper Spectra) though not from each other. In the GCxGC plot shown in Figure 7 both compounds are clearly separated from the column bleed and each other allowing for easy, automatic detection and identification of the previously hidden pesticide.

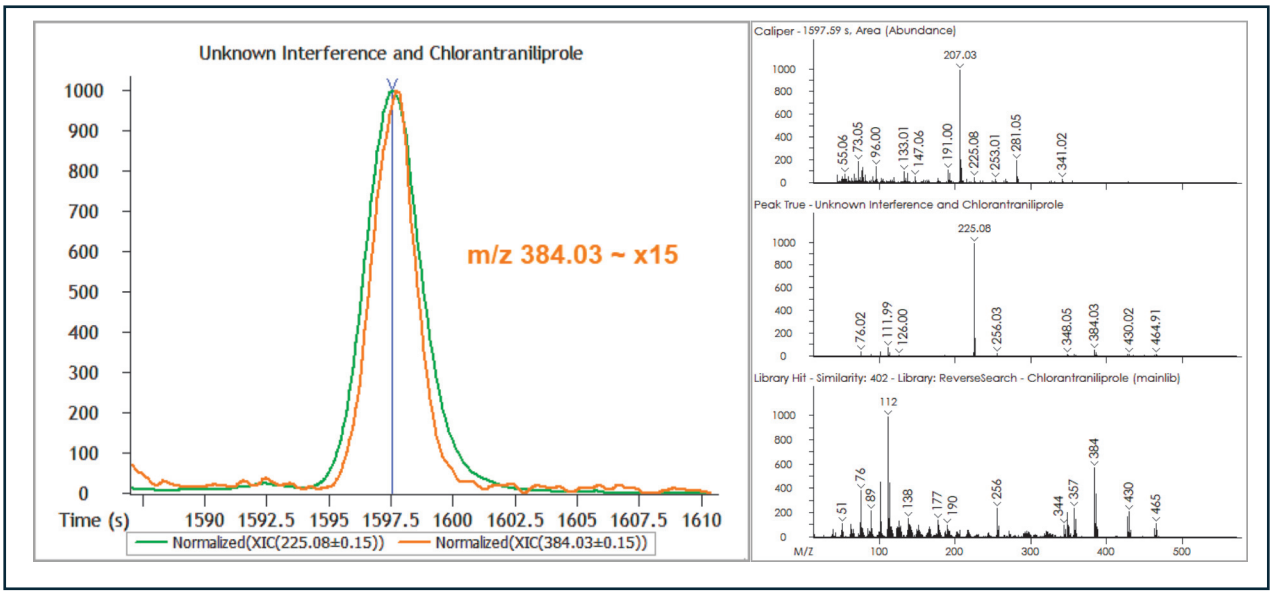

Figure 6. GC Extracted Ion Chromatogram (XIC) and Spectra plots of the Chlorantraniliprole and the interference compound. The two compound signals have been normalized to allow for easier viewing. The top, raw spectra plot (Caliper) shows the intensity of both compounds relative to the overriding column bleed signal. In the middle deconvoluted (Peak True) spectra you can see the most prevalent ions from Chlorantraniliprole though they are obviously dwarfed by ions from the coeluting, matrix compound.

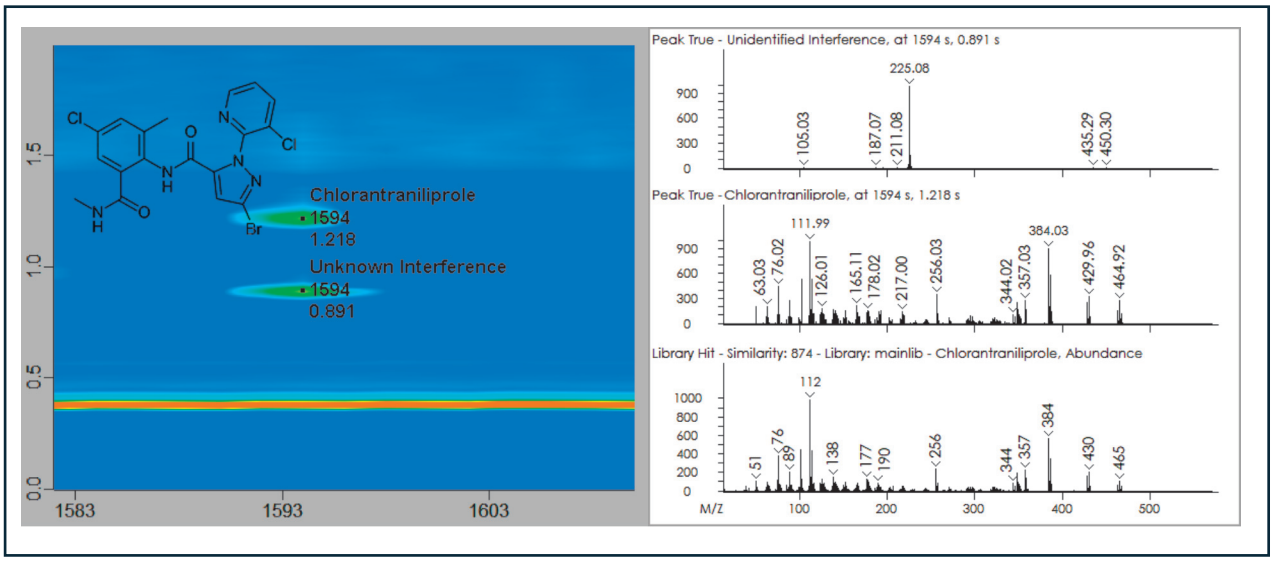

Figure 7. GCxGC Contour & Spectral plots of Chlorantraniliprole and the interference compound. The two compound signals have been normalized to allow for easier viewing. Note the separation from the column bleed (horizontal band) and the improvement in the deconvolution of both compound spectra compared to their GC results in Figure 6.

Conclusion

Pegasus BT 4D's exceptional sensitivity is further enhanced by increased chromatographic resolution offered by GCxGC separations. The increased chromatographic resolution allows for better selectivity, peak detection and identification, improved linearity, and more confidence in quantitation for both targeted and untargeted compounds especially, in samples with complex matrices. The GCxGC results easily met and exceeded the SANTE 2017 requirements.

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