GCxGC-TOFMS OF SYNTHETIC PYRETHROIDS IN FOOD

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Introduction

Pyrethrins are natural insecticides in the extract of chrysanthemum flowers[1]. Pyrethroids are synthetic forms of pyrethrins, and many are halogenated (F, Cl, Br). Synthetic pyrethroids have become popular replacements for organophosphorus pesticides, which have become increasingly regulated due to health and environmental concerns. Synthetic pyrethroids were designed to be more stable than pyrethrins, and therefore have the potential to be found in the environment, and in food, as trace residues. They are highly toxic to aquatic organisms and some may be endocrine disruptors and/or carcinogens. Typical methods for their analysis include gas chromatography – electron capture detector (GC-ECD)[2], GC – electrolytic conductivity detector (ELCD)[3], and GC – mass spectrometry (MS)[4].

A relatively new way to solve separation problems for complex samples, including those for food analysis, is to use comprehensive two-dimensional GC (GCxGC). GCxGC increases peak capacity by applying two independent separations to a sample in one analysis. Typically, GCxGC involves a serial column configuration (employing orthogonal phases) separated by a thermal modulator. Due to modulation, most GCxGC peaks are on the order of 50 to 250 ms wide, requiring a fast detector. When mass spectrometry is used, only time-of-flight (TOF) has the necessary acquisition rates (hundreds of spectra/sec). The ability of the thermal modulator to narrow peaks (thereby increasing their height) prior to their detection also affords the ability to increase TOFMS sensitivity, which can be important for the analysis of trace levels of pesticides in food samples, not only to increase their detection, but also to allow moderate (1 to a few microliters) sample sizes to be introduced to the GC. Keeping the sample introduction volume low, especially in food analysis, helps preserve the integrity of the chromatographic system.

GCxGC with a flame ionization detector (FID) was recently employed for the analysis of pyrethrins (cinerins, jasmolins, pyrethrins) in a chrysanthemum extract[5]. Since these pyrethrins are thermally labile, on-column injection and, very short primary and secondary columns were used. Fast GCxGC with a short primary column allowed the unbiased determination of the pyrethrins in the complex extract, but the technique was not used for synthetic pyrethroid analysis. The paper offered here demonstrates GCxGC-TOFMS for the analysis of synthetic pyrethroids, with an emphasis on those that contain halogens. A pyrethroid-spiked composite food extract is used to demonstrate the utility of GCxGC-TOFMS for complex matrices.

Materials and Methods

Standard solutions in ethyl acetate containing 50, 20, 10, and 5 pg/µL of synthetic pyrethroids were used for calibration. Separations were carried out using a LECO Pegasus 4D GCxGC-TOFMS that has a quad-jet, dual-stage modulator (St. Joseph, MI, USA). Both columns for the GCxGC configuration were from Restek Corporation (Bellefonte, PA, USA). The primary column, a 27m x 0.25mm x 0.25µm Rtx-1, was press-fitted to a 1m x 0.10mm x 0.10µm Rtx-CLPesticides2 residing in a secondary oven that can be independently temperature programmed versus the primary oven. Modulation was on the Rtx-CLPesticides2 column. A 1 µL splitless injection at 250°C, with a purge time of 60 sec, was used for each analysis. The primary oven was programmed as follows: 45°C (1 min), 40°/min to 125°, 5°/min to 315°. The modulator temperature offset was 30°C. The second dimension separation

time (modulation time) was set to 4 sec, with hot pulse time and cool time between stages at 1 sec each. The secondary oven program was: 50°C (1 min), 40°/min to 130°, 5°/min to 320°. Helium carrier gas was a constant 2.5 mL/min. Total run time was 41 min. Electron ionization at 70eV was used for TOFMS with a source temperature of 225°C, a data acquisition rate of 100 spectra/sec, and a stored mass range of 45 to 550 u.

To evaluate the performance of GCxGC-TOFMS for pyrethroids in food, spiked medium fat composite diet extracts were analyzed, with the spike levels being the same as the standard concentrations.

Results and Discussion

Figure 1 shows a contour plot for a mix of synthetic pyrethroids with internal standards and a surrogate pesticide. Note that compounds are separated not only in the first dimension (the Rtx-1 column), but also in the second dimension (Rtx-CLPesticides). For the cyfluthrins and cypermethrins, which have four isomers each in the standard mix, three peaks for each group were seen (the last two in each set coeluted).

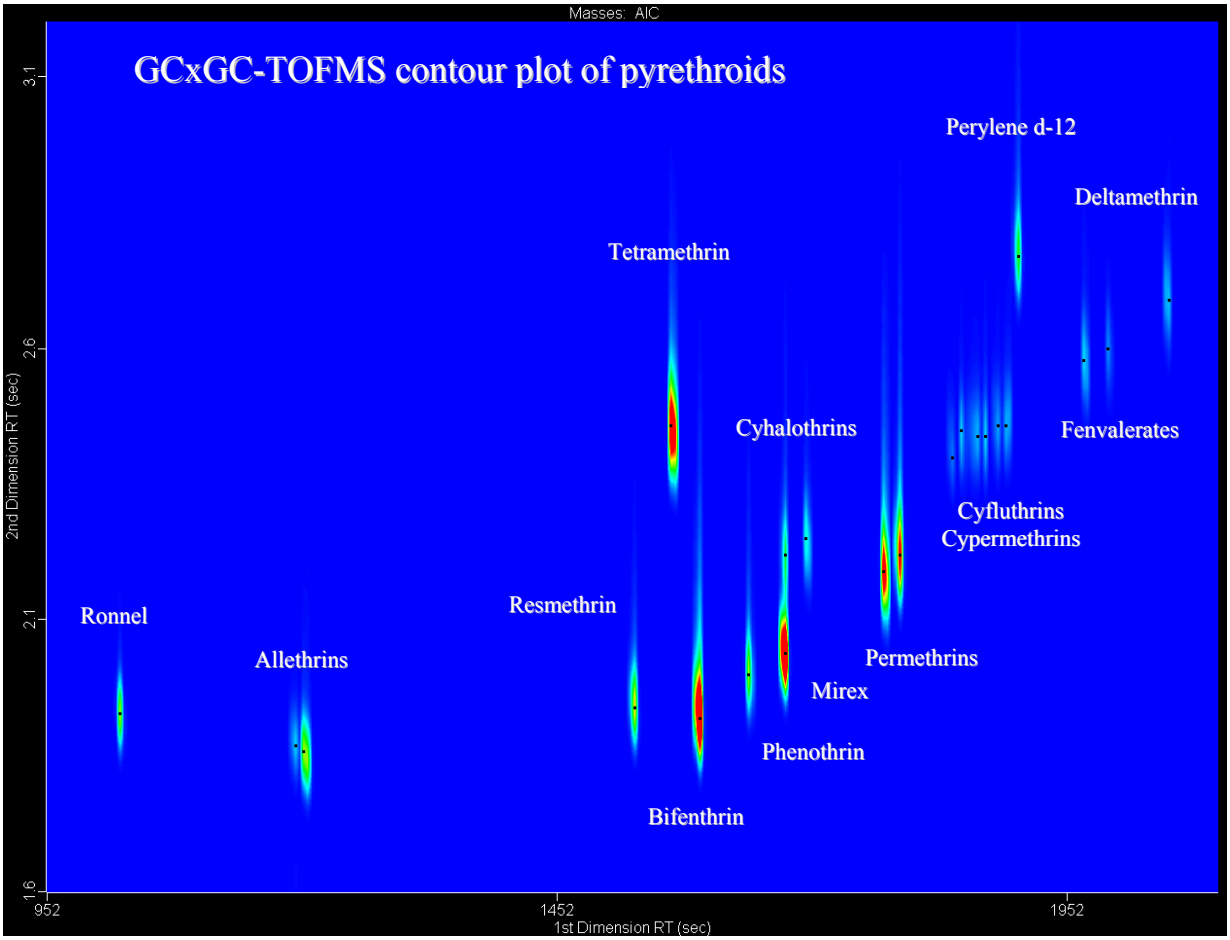

Figure 1. GCxGC contour plot of some synthetic pyrethroids on the Rtx-1 x Rtx-CLPesticides2 column set. Ronnel, Mirex, and Perylene d-12 are not pyrethroids, and are being used as internal standards and a surrogate. This contour plot only represents part of the whole chromatographic run in both dimensions.

Figure 2 is a contour plot that demonstrates the power of GCxGC to eliminate interferences that commonly confound the quantitative analysis of pesticides, including pyrethroids, in food matrices. Even mass spectrometry, if used with only a one-dimensional GC method, would be no help at resolving food matrix interferences and Bifenthrin.

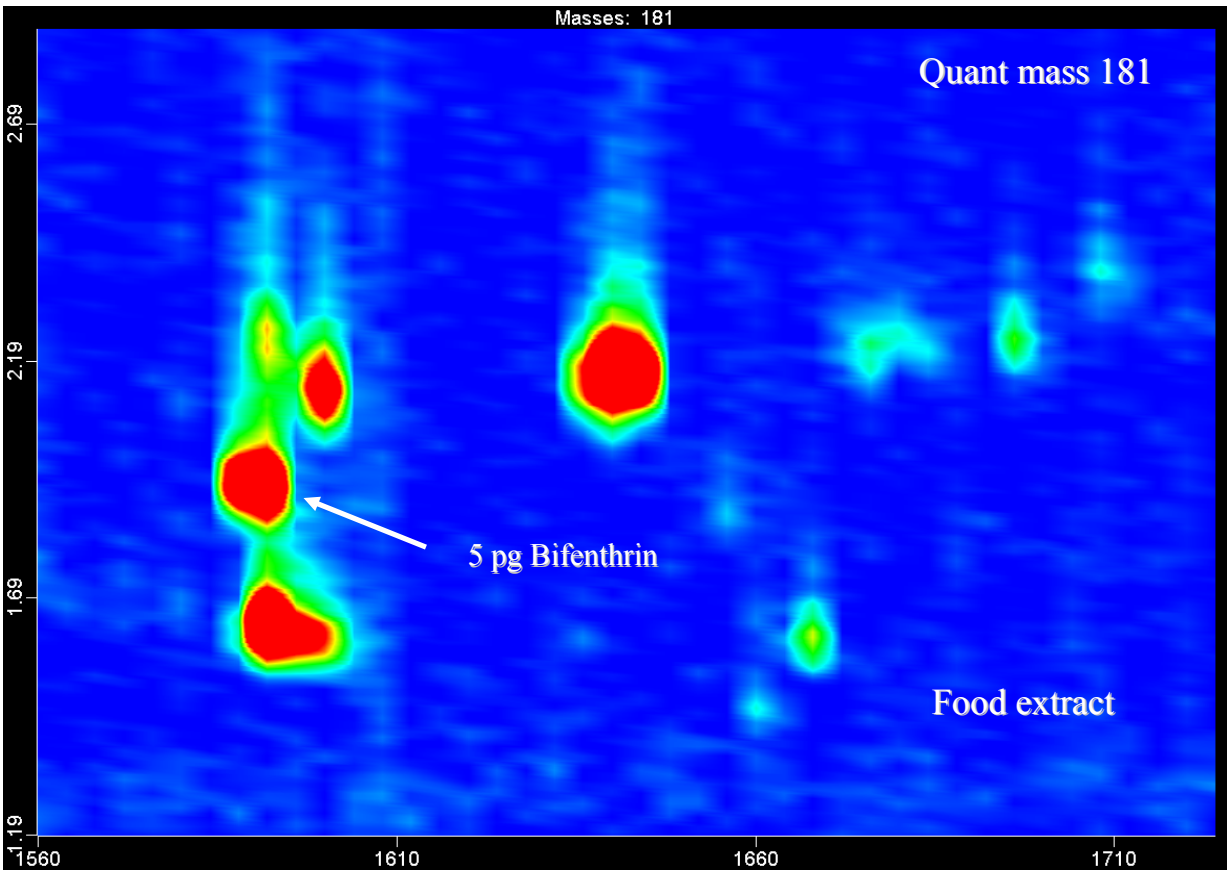

Figure 2. GCxGC contour plot for the quantification mass of Bifenthrin. The peaks (spots) above and below the Bifenthrin peak would be overwhelming interferences, both for qualitative identification, and quantification, if only a one-dimensional GC-MS analysis was used. Bifenthrin is separated from these interferences though when using GCxGC.

Table 1 shows the GCxGC-TOFMS quantification results for the food matrix extracts that had been spiked with pyrethroids. These results were obtained from calibration curves composed from the 5-50 pg/µL standards. The internal standard method was used for calibration and quantification, and a single ion was used for each compound as a quantification mass. For the pyrethroids whose standards consist of isomeric mixes (allethrin, cyfluthrin, cypermethrin), a group value is reported here. In general, the GCxGC-TOFMS quantified results for the spiked extracts are very good, being close to the expected values. An exception is Deltamethrin, whose values are likely corrupted by the presence of Tralomethrin, in both standards and spikes, which thermally degrades in hot injection ports to Deltamethrin.

Although the focus of this work was for the halogenated synthetic pyrethroids, the peak capacity increase afforded when using GCxGC-TOFMS offers an important advantage for determining other pyrethroids like Allethrin and Resmethrin. The low m/z ions used for their quantification are easily biased by compounds normally present in food extracts when using one-dimensional GC-MS. The results in **Table 1** indicate that was not a problem with GCxGC-TOFMS.

Table 1. GCxGC-TOFMS quantified results for analysis of synthetic pyrethroids in medium fat diet composite extracts. The column headers in red indicate the names for the compounds of interest, including Mirex, a surrogate, the quantification mass, and the pg/µL amounts for the spikes. Pyrethroids highlighted in blue are halogenated. ND is "not detected".

Name	Q Mass		50		20		10		5
Allethrin	123		55		23		15		4.8
Resmethrin	123		55		24		12		5.5
Tetramethrin	164		51		20		9.0		4.4
Bifenthrin	181		50		20		8.2		4.7
Phenothrin	183		49		20		11		5.8
Mirex	272		48		22		9.3		4.8
Cyhalothrin 1	181		52		27		8.6		4.6
Cyhalothrin 2	181		47		24		10		4.4
cis-Permethrin	183		51		25		9.5		4.9
trans-Permethrin	183		47		26		9.6		5.2
Cyfluthrin	163		54		24		12		2.4
Cypermethrin	163		47		20		8.6		4.9
Fenvalerate 1	167		42		19		10		6.1
Fenvalerate 2	167		52		21		9.1		ND
Deltamethrin	181		110		24		38		9.5

Conclusions

GCxGC-TOFMS is a powerful method for qualitatively identifying, and then quantifying, synthetic pyrethroids and other pesticides in food matrices. GCxGC-TOFMS sensitivity for many of the halogenated pyrethroids is at least in the low pg range, which makes it attractive for trace level determinations without resorting to the complications of large volume injection techniques.

Disclaimer

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute EPA endorsement or recommendation for use.

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