Novel Analysis Method of Various Environmental Samples Using GC×GC-HRMS with Encoded Frequent Pulsing™ (EFP™)

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Introduction

Environmental contaminants are a diverse group of compounds that come with many challenges when it comes to detecting and properly identifying each of the various compound groups. Add in the complications due to sample matrix interference, and confidently identifying environmental contaminants within a sample matrix can be a formidable task. By combining the separation power of comprehensive two-dimensional gas chromatography with a high resolution multi-reflecting TOFMS that uses Encoded Frequent Pulsing (EFP™) technology, the ability to analyze samples with a resolving power greater than 25,000, sub-ppm mass accuracies, acquisition rates up to 200 spectra-per-second, and sub-picogram limits of detection is possible.

The addition of EFP technology with specialized real-time decoding algorithms provides the ability to increase the extraction frequency of the instrument, thus increasing the duty-cycle without sacrificing spectral performance. To evaluate the performance of this new technology, a typical set of performance standards was first tested with and without EFP enabled to develop a baseline for sensitivity testing using prototype instrumentation. Then a complex mix of environmental standards was tested to evaluate the sensitivity of the instrument with regard to various compound types. This was then followed by the analysis of a set of pesticide residue standards in matrix to test 'real-world' performance using comprehensive two-dimensional gas chromatography coupled with a high resolution multi-reflecting TOFMS prototype with EFP.

Challenges

The Pegasus® GC-HRT is a High Resolution Multi-Reflecting TOFMS. This technology achieves a resolving power > 50,000, sub-ppm mass accuracies, acquisition rates up to 200 spectra/sec, excellent isotope ratio fidelity, and a wide mass range. This is made possible because of a long flight path (20 m), a long flight time (1 ms), and thus a low duty-cycle. This design also uses an orthogonal pulser without a trap, which means that there will be ions that are lost during the interval between pulses which may affect its sensitivity.

EFP is a novel multiplexing approach which allows for an increase in sensitivity by increasing the pulser frequency without overlapping m/z peaks in the resulted mass spectra. The feature that separates this approach to multiplexing from other approaches is the use of unequal pulse intervals. It is this unequal pulse interval that prevents the possible loss in sensitivity due to overlapping m/z peaks in the analyzer. By knowing the sequence of the pulse intervals, decoding of the signal is possible. Figure 1 shows an example of the unequal pulse intervals created by the pulser. Figure 2 shows the resultant non-decoded EFP spectra. Figure 3 shows a comparison of a single analyte spectrum not using EFP to one using EFP. There is also a spectral plot of the non-decoded spectra that is collected and decoded behind the scenes for the resultant spectrum displayed.

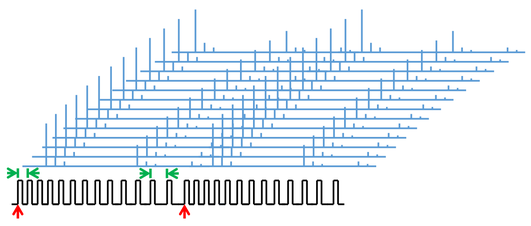

Figure 1. Example of unequal pulser intervals used in EFP.

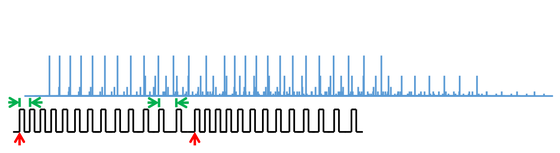

Figure 2. Example of a non-decoded EFP spectra.

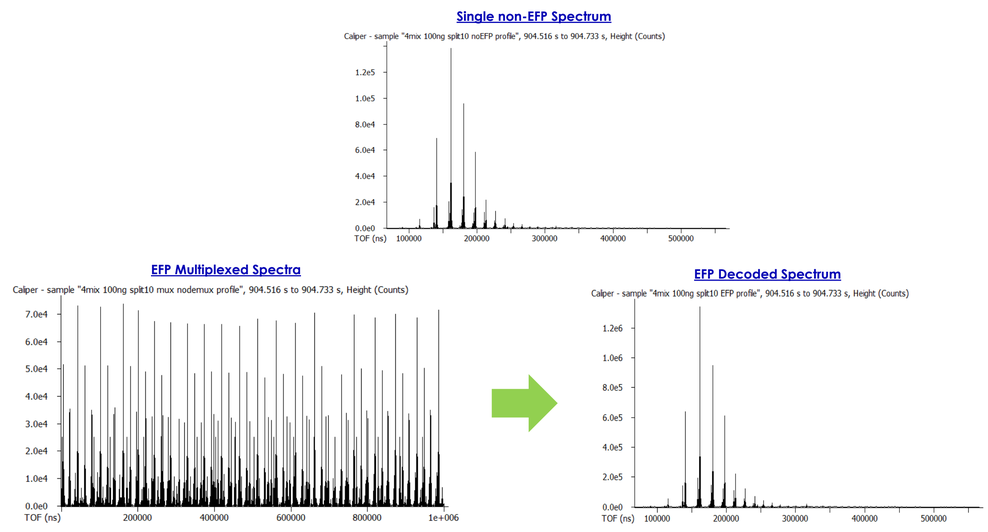

Figure 3. Spectral plot of a non-EFP analyte spectrum, a plot of a non-decoded EFP multiplexed spectra, and a plot of the decoded spectrum.

Experimental

A set of eight injections of 1 pg/µL of OFN were collected without the use of EFP. This resulted in an IDL calculation of 0.12 pg/µL. Then another set of eight injections of 0.10 pg/µL OFN were collected using EFP which gave an IDL calculation of 0.04 pg/µL. This was then followed by another eight injections of 0.05 pg/µL OFN which gave an IDL calculation of 0.02 pg/µL. Figure 4 shows overlapped chromatographic plots of m/z 271.98 for each of the individual sets of experiments demonstrating the response for each of the sets of data.

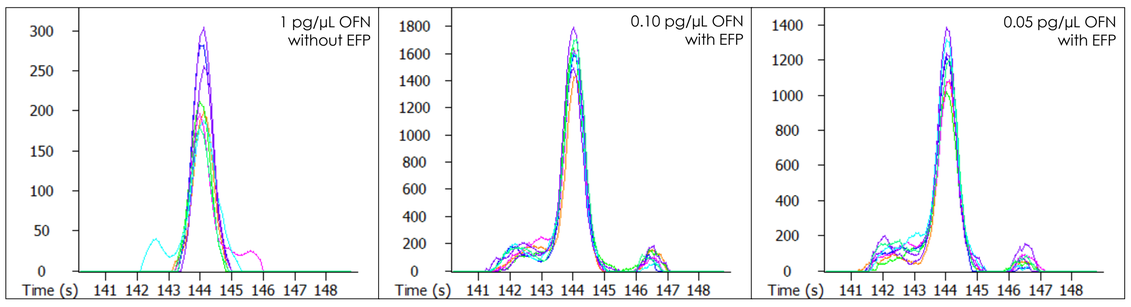

Figure 4. Overlapped chromatographic plots displaying m/z 271.98 of 1 pg/µL OFN that is not using EFP and 0.10 pg/µL OFN and 0.05 pg/µl OFN both using EFP.

Sensitivity can be demonstrated with a standard performance standard, but the next step is to see how well EFP behaves with a complex mix of environmental standards. A set of 15 environmental standard mixes, purchased separately from Restek Corporation, were mixed together creating a sample containing 317 compounds of various compound classes. The standard was then analyzed one-dimentionally at 8 spectra/sec and then at 200 spectra/sec for a comprehensive GC×GC analysis. Figure 5 shows a contour plot of the 200 spectra/sec GC×GC run.

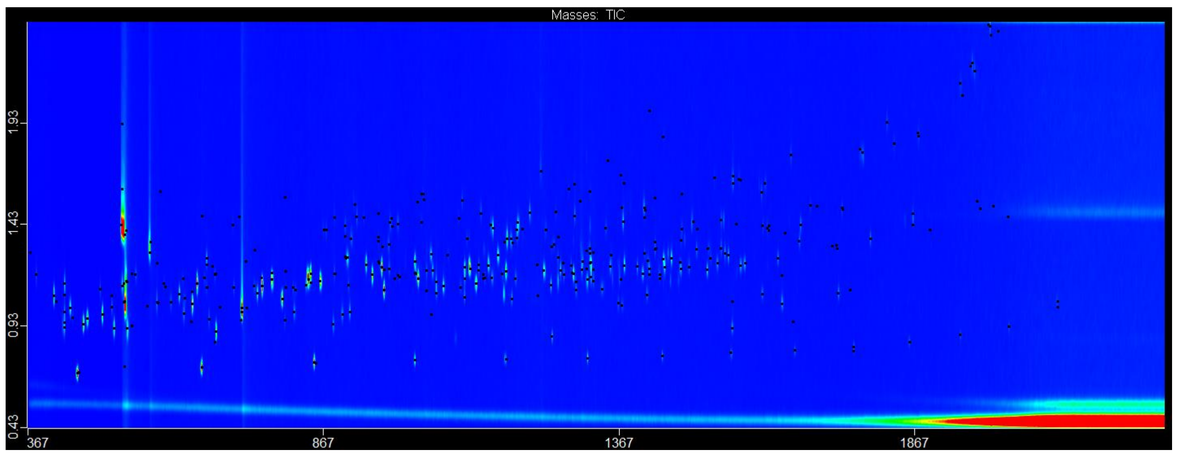

Figure 5. The above is a contour plot displaying the TIC of the 100 pg/µL on-column complex mix analyzed in split mode.

Zooming into a section of the contour plot shows that the use of EFP does not compromise the separation power of GC×GC in a complex sample. Figure 6 shows a zoomed-in portion of both the 8 spectra/sec one-dimentional run and the 200 spectra/sec GC×GC run contour plot.

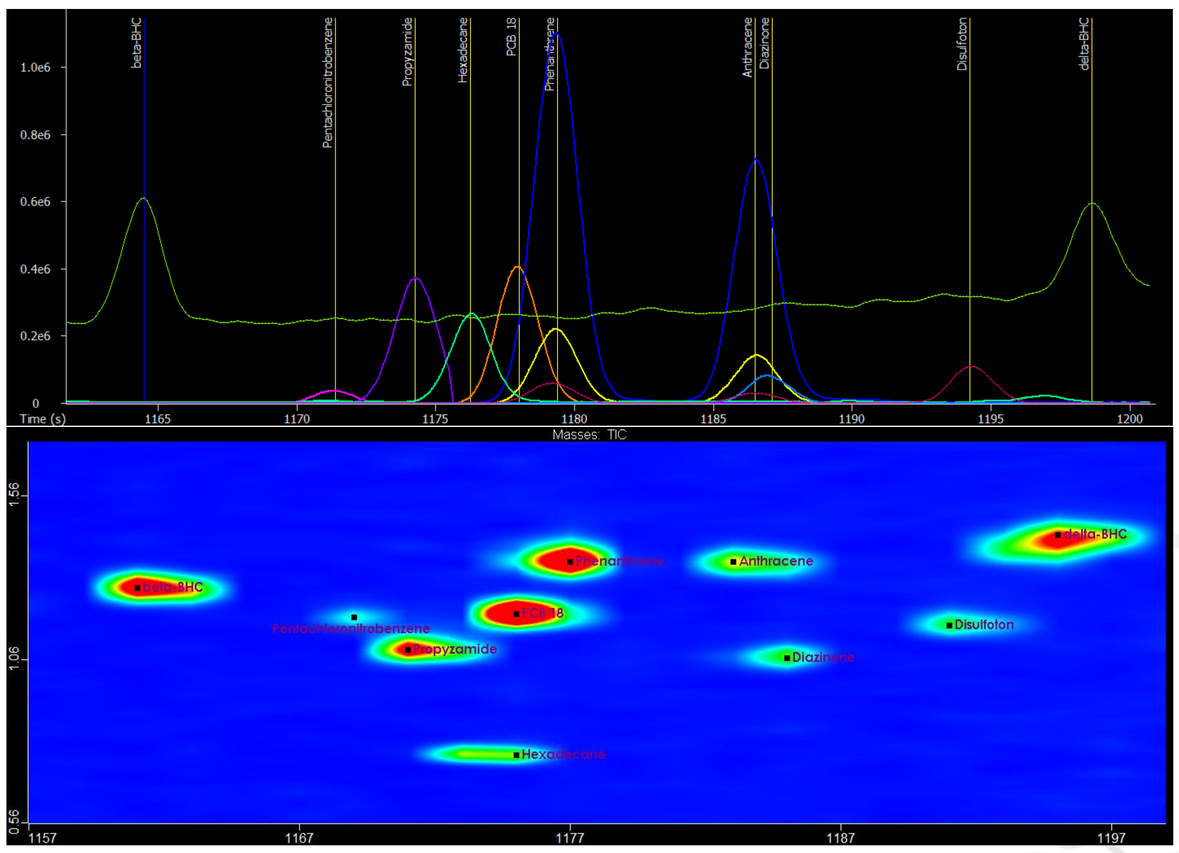

Figure 6. The above is a one-dimensional chromatographic plot of a zoomed-in portion of the complex environmental standard. The contour plot below it is a zoomed-in section of the same complex environmental standard showing the same set of analytes.

Figure 7 is a zoomed-in contour plot of the 200 spectra/sec GC×GC run showing the same analytes at a 5 pg/µL concentration on-column using a split injection. Figure 8 shows two zoomed-in chromatographic plots of pesticide examples in a 8 spectra/sec analysis of an eggplant pesticide residue sample that has been spiked with various standards at a 5 pg/µL concentration to demonstrate the instruments capability analyzing pesticides in matrix.

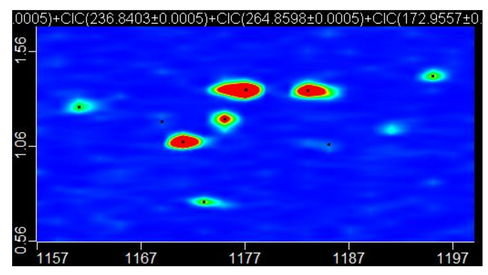

Figure 7. Contour plot of a 5 pg/µL on-column concentration of a complex standard mix collected in split mode.

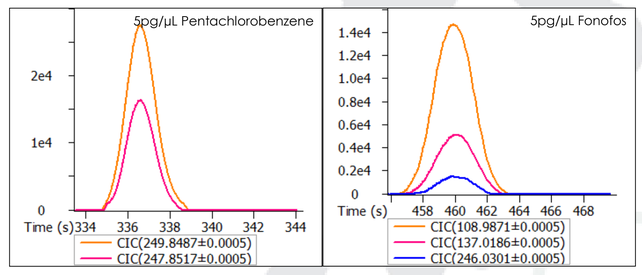

Figure 8. Chromatographic plots of 2 pesticides in an eggplant pesticide residue sample.

Conclusion

The use of a novel multiplexing approach called Encoded Frequent Pulsing has demonstrated the ability to increase the sensitivity of a High Resolution Multi-Reflecting TOFMS without compromising other aspects of its performance. This new approach has the potential to make the benefits of a HR-TOFMS capable of 200 spectra/sec available to new applications that need extra sensitivity along with sub-ppm mass accuracies and a resolving power greater than 25,000.

Acknowledgements

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