Novel Residue Analysis of Various Food Samples using GC- and GC×GC-HRMS with Encoded Frequent Pulsing™

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Introduction

Environmental contaminants are a diverse group of compounds that come with many challenges when it comes to detecting, properly identifying, and quantifying 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 Folded Flight Path® (FFP®) TOFMS that uses Encoded Frequent Pulsing (EFP™) technology, the ability to analyze samples with a full mass range 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 full mass range resolving power >25,000, with sub-ppm mass accuracies, acquisition rates up to 200 spectra/sec, and excellent isotope ratio fidelity. 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's this unequal pulse interval that prevents the possible loss in sensitivity due to overlapping m/z peaks in the analyzer.[1]

Experiments

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 1 shows overlapped chromatographic plots of m/z 271.9867 ± 5 mDa for each of the individual sets of experiments demonstrating the response for each of the sets of data.

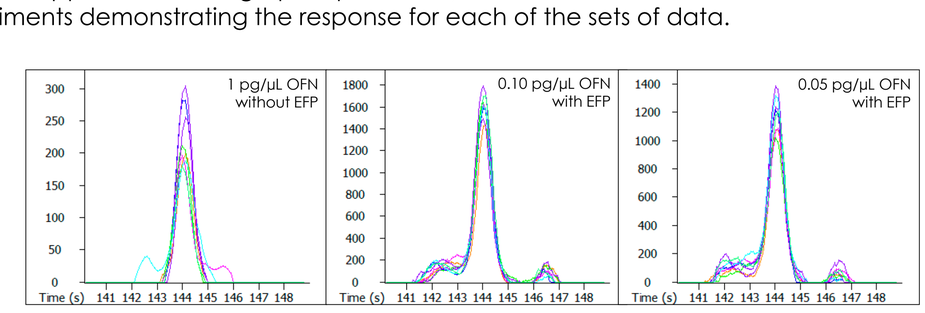

Figure 1. Overlapped chromatographic plots displaying m/z 271.98 ±5 mDa of 1 pg/µL OFN that is not using EFP and 0.10 pg/µL OFN and 0.05 pg/µl OFN both using EFP.

Sensitivity can be demonstrated with a typical performance standard, but the next step is to see how well EFP behaves with a set of environmental standards in matrix. A set of six calibration standards containing 107 pesticides in an eggplant matrix were prepared at concentrations ranging from 0.5 ppb to 20 ppb. Figure 2 shows a chromatographic plot displaying the TIC of the 10 ppb standard along with a chromatographic plot displaying the quant masses (AIC) for each of the analytes in the sample.

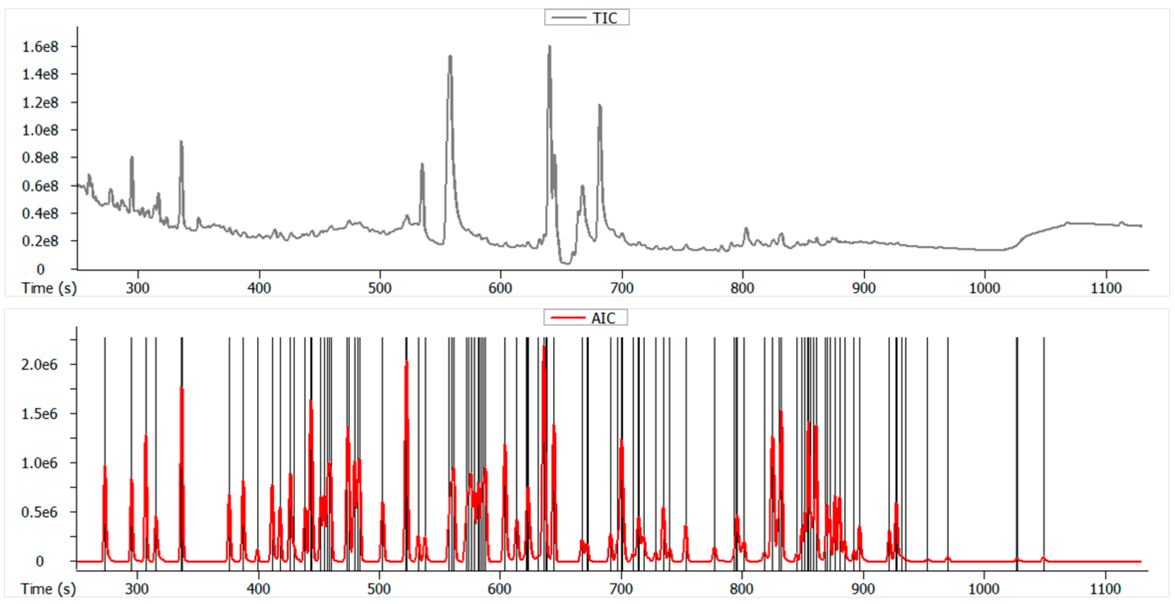

Figure 2. Chromatographic plot of a 10 ppb standard in an eggplant matrix displaying the TIC above and the AIC below.

The instrument is able to produce very good spectral quality at the required low concentrations. Figure 3 shows a couple examples of the spectral quality of a-BHC and Tefluthrine at 10 ppb in an eggplant matrix displaying their caliper spectrum, deconvoluted (Peak True) spectrum, and library match spectrum. The a-BHC has a similarity match of 894 and the Tefluthrine has a similarity match of 929.

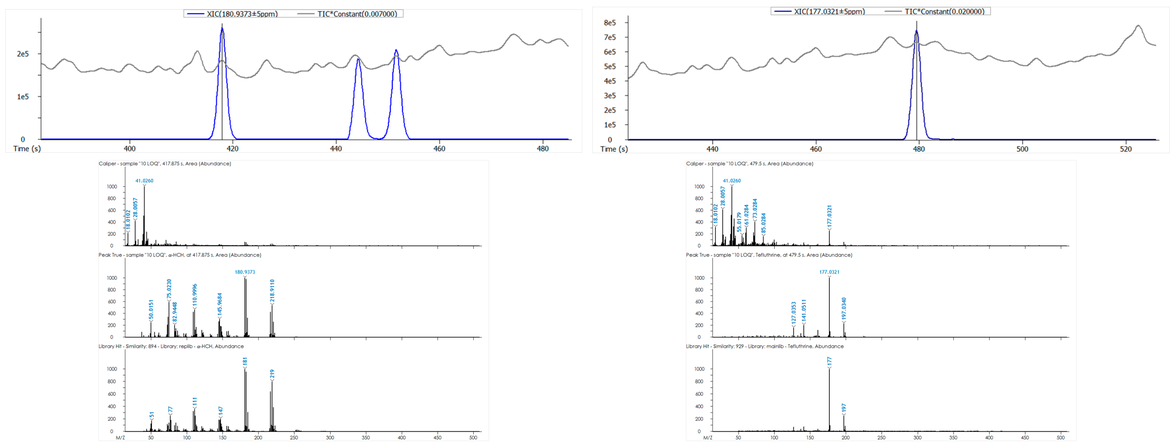

Figure 3. Zoomed in chromatographic plots of a-BHC and Tefluthrine with their respective spectral plots below.

The instrument shows very good linearity for the calibration levels tested. Table 1 displays the calibration table along with their associated LOD and the coefficient of determination values (R^2) associated with their individual calibration curves.

Table 1. Pesticide Calibration Table ranging from 0.5 ppb to 20 ppb

Name	R.T. (s)	Quant Mass (± 5 ppm)	R^2	LOD (ppb)
Dichlobenil	273.1	170.9637	0.99948	0.075
Mevinphos	295.1	192.0182	0.99952	0.083
Pebulate	306.9	128.1069	0.99820	0.052
Etridiazole	307.3	182.9181	0.99814	0.052
2,4-Dimethylphenyl formamide	315.4	149.0835	0.99822	0.079
Pentachlorobenzene	336.6	249.8486	0.99886	0.054
Tecnazene	336.8	214.8797	0.99780	0.136
Diphenylamine	376.0	169.0886	0.99800	0.200
Chlorpropham	387.4	213.0551	0.99858	0.120
Trifluralin	399.3	306.0696	0.99531	0.500
Phorate	411.4	121.0412	0.99646	0.188
BHC alpha	417.9	180.9373	0.99612	0.214
Hexachlorobenzene	426.5	283.8096	0.99728	0.150
Dicloran	429.1	205.9644	0.99830	0.682
Atrazine	438.8	215.0932	0.99864	0.300
Clomazone	443.1	204.1019	0.99702	0.150
BHC beta	444.3	180.9373	0.99776	0.375
Lindane	451.6	180.9373	0.99610	0.375
Terbuthylazine	454.4	214.0854	0.99734	0.375
Pentachloronitrobenzene	457.1	294.8337	0.99226	0.375
Pronamid	458.3	172.9555	0.99634	0.300
Fonofos	459.9	109.0106	0.99904	0.167
Disulfoton	473.4	96.9509	0.99768	0.094
Terbacil	474.5	160.0034	0.99850	0.375
Tefluthrine	479.5	177.0321	0.99884	0.088
Chlorothalonil	481.9	265.8780	0.99902	0.150
Triallate	483.6	268.0324	0.99838	0.054
Pentachloroaniline	502.8	264.8595	0.99712	0.125
Vinclozoline	522.0	284.9954	0.99906	0.300
Chlorpyrifos methyl	522.5	287.9226	0.99856	0.068
Parathion methyl	522.5	263.0011	0.99726	0.115
Heptachlor	532.1	100.0074	0.99710	0.500
Metalaxyl	537.6	206.1175	0.99856	0.214
Pirimiphos methyl	557.5	305.0957	0.98595	0.094
Linuron	559.5	159.9715	0.99618	0.563
Methyl pentachlorophenyl sulfide	561.5	295.8362	0.99800	0.250
Anthraquinone	572.0	208.0518	0.99694	0.197
Aldrin	573.4	262.8564	0.99678	1.200
Metolachlor	575.8	238.0993	0.99686	0.136
Fenthion	578.6	278.0194	0.99776	0.130
Chlorpyrifos	581.6	196.9196	0.99798	0.091
Parathion ethyl	582.4	125.0293	0.99818	0.333
Dicofol	584.3	110.9996	0.99852	0.375
Triadimefon	585.5	208.0285	0.99513	0.600
DCPA	587.8	300.8802	0.99726	0.130
Diphenamid	603.8	72.0443	0.99842	0.058
Cyprodinil	613.6	224.1182	0.99572	0.214
Pendimethalin	621.8	119.0603	0.99634	1.071
Heptachlor epoxide	622.5	352.8436	0.99686	0.429
Penconazole	623.3	158.9762	0.99475	0.600
Captan	631.4	149.0471	0.99549	4.286
Fipronil	635.9	212.9480	0.99758	0.750
Allethrin	635.9	123.1168	0.99554	0.333
Quinalphos	636.4	146.0474	0.99578	0.300
Triadimenol	637.5	112.0505	0.99445	0.167
Folpet	638.9	259.9334	0.99525	2.813
Procymidone	644.3	283.0161	0.99786	0.250
Endosulfan I	667.6	159.9841	0.99920	0.500
Chlordane (cis)	671.6	372.8254	0.99523	0.300
Prothiofos	691.1	161.9633	0.99592	0.300
Fludioxonil	697.5	248.0391	0.99449	0.300
DDE(p,p')	699.8	317.9345	0.99501	0.167
Dieldrin	701.1	262.8564	0.99664	0.500
Myclobutanil	709.6	179.0622	0.99832	0.682
Oxyfluorfen	713.8	302.0190	0.99680	0.938
Flusilazole	714.4	233.0592	0.99455	0.300
Bupirimate	718.6	316.1563	0.99916	0.375
Endrin	728.4	262.8564	0.99828	1.500
Chlorfenapyr	734.6	59.0491	0.99766	0.115
Endosulfan II	740.1	236.8413	0.99746	1.250
DDD(p,p')	753.3	235.0075	0.99507	0.200
Triazophos	777.0	161.0583	0.99610	0.600
Carfentrazone ethyl	793.3	330.0251	0.99806	1.000
Endosulfan sulfate	795.3	271.8096	0.99568	0.536
Lenacil	796.0	136.0393	0.99556	0.136
DDT(p,p')	801.5	235.0075	0.99856	0.333
Tebuconazole	818.4	125.0152	0.99656	0.375
Propargite	825.0	135.0804	0.99485	0.500
Piperonyl butoxide	830.1	176.0831	0.99682	0.167
Resmethrin	832.3	123.1168	0.99435	0.667
Iprodione	844.9	314.0093	0.99932	0.625
Phosmet	849.1	160.0393	0.99409	0.250
Bromopropylate	851.8	340.8994	0.99612	0.333
Tetramethrin	854.4	123.1168	0.99806	0.200
Bifenthrin	854.8	165.0698	0.99523	0.115
Methoxychlor	856.8	227.1066	0.99572	0.200
Fenpropathrin	858.8	125.0960	0.99267	0.600
Tebufenpyrad	861.0	333.1602	0.99608	0.500
Phenothrin cis	868.5	123.1168	0.99333	1.071
Tetradifon	870.0	355.8808	0.99582	0.214
Phenothrin	872.3	168.0569	0.99738	0.333
Phosalone	876.6	182.0003	0.99467	0.375
Azinphos-methyl	876.6	160.0505	0.99036	0.800
Pyriproxyfen	880.4	136.0756	0.99754	0.107
Cyhalothrin	884.6	181.0647	0.99734	0.321
Cyhalothrin Isomer	892.4	181.0647	0.99932	0.900
Fenarimol	896.9	107.0239	0.99770	0.214
Permethrin (cis)	921.6	163.0075	0.99870	0.125
Permethrin (trans)	927.1	163.0075	0.99363	0.125
Pyridaben	927.5	147.1168	0.99564	0.231
Coumaphos	931.8	362.0139	0.99948	2.143
Prochloraz	934.6	70.0287	0.99578	2.500
Cyfluthrin, total	958.3	163.0075	0.99235	2.500
Cypermethrin, total	969.9	163.0075	0.99814	2.000
Fluvalinate	1026.9	250.0604	0.99297	2.500
Fenvalerate	1027.6	125.0152	0.99744	1.500
Deltamethrin	1048.9	181.0647	0.99890	1.250

Comprehensive two-dimensional chromatography was then applied to the same sample set with slightly different methodology to show the benefits of separating out the sample matrix from the analytes of interest. Figure 4 shows the one-dimensional TIC plot of a 2.5 ppb standard in an eggplant matrix. Figure 5 shows a zoomed in plot highlighting a-BHC and Tefluthrine. In this example, the a-BHC is obstructed by a strong siloxane peak.

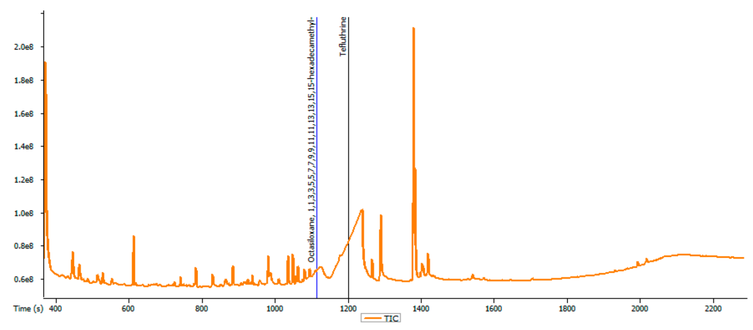

Figure 4. One-dimensional TIC chromatographic plot of a 2.5 ppb eggplant standard.

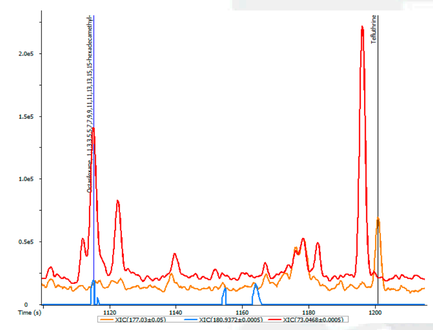

Figure 5. Zoomed in plot of a 2.5 ppb eggplant standard.

Figure 6 shows a GCxGC contour plot displaying the TIC of the same 2.5 ppb eggplant standard. Figure 7 shows a zoomed in portion of the 2.5 ppb eggplant standard highlighting the separation of the a-BHC from the siloxane peak and the Tefluthrine from the sample matrix.

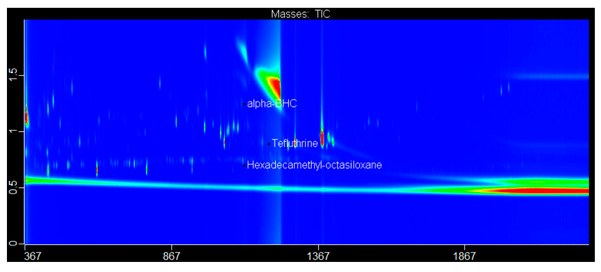

Figure 6. GCxGC contour plot displaying the TIC of a 2.5 ppb eggplant standard.

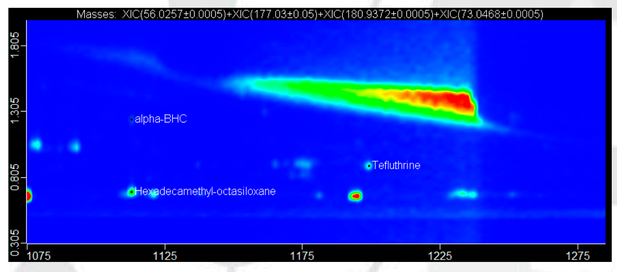

Figure 7. Zoomed in GCxGC contour plot highlighting the separation of analytes of interest from interfering peaks in a 2.5 ppb eggplant standard.

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 bring the benefits of a HR-TOFMS coupled with GC or GCxGC to applications that need extra sensitivity along with sub-ppm mass accuracies and a full mass range resolving power greater than 25,000 for better confidence in peak identification at present and in future retrospective analysis of complex samples.

Acknowledgements

We would like to thank Raymond Allum from the Florida Department of Agriculture and Consumer Services for providing sample extracts for our testing.

References

[1]ASMS 2015 Proceedings, TOA am, P. Willis et al. *High Resolution Multi-Reflecting TOFMS with Multiplexing by Encoded Frequent Pulsing for Increasing the Duty Cycle 10 to 100 Times.*

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