Fast Analysis of 140 Environmental Compounds by GC/MS/MS

Erinn O'Neill[1], Alexis Willey[1], and Marta Venier[2]
[1]Agilent Technologies Inc., Wilmington, Delaware, USA
[2]Indiana University, Bloomington, Indiana, USA

INDIANA UNIVERSITY

Agilent

Introduction

Pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) are persistent environmental pollutants with the potential for bioaccumulation.[1] Previously, these compound classes have been analyzed using gas chromatography with an electron capture detector (GC-ECD), but this requires full chromatographic separation of all analytes for confident chemical identification. With a triple quadrupole detector (QQQ), analysis time significantly drops due to mass identification eliminating the need to chromatographically separate all components. Further, multiple reaction monitoring (MRM) mode on the QQQ provides a higher confidence in peak identification than analysis by scan or selected ion monitoring (SIM) mode. Combining chromatographic separation and MS/MS analysis is especially useful for PAHs and PCBs where multiple analytes may have the same m/z value.

PAH	Conc. (ppb)
Acenaphthene	200
Acenaphthylene	200
Anthracene	200
Benz[a]anthracene	200
Benzo[b]fluoranthene	200
Benzo[k]fluoranthene	200
Benzo[g,h,i]perylene	200
Benzo[a]pyrene	200
Benzo[e]pyrene	200
Chrysene	200
Coronene	200
Dibenz[a,h]anthracene	200
Fluoranthene	200
Fluorene	200
Indeno[1,2,3-cd]pyrene	200
Naphthalene	200
Phenanthrene	200
Pyrene	200
Retene	200
Perylene-d_{12}	200
Benz[a]anthracene-d_{12}	200
Anthracene-d_{10}	200
Phenanthrene-d_{10}	200
Pyrene-d_{10}	200

Pesticide	Conc. (ppb)
α-HCH	20
β-HCH	20
γ-HCH	20
δ-HCH	20
ε-HCH	20
Heptachloroepoxide	20
Aldrin	20
Dieldrin	20
Endrin	20
p,p'-DDT	20
p,p'-DDD	20
p,p'-DDE	20
o,p'-DDD	20
o,p'-DDT	20
α-Chlordane	20
γ-Chlordane	20
Oxychlordane	20
trans-Nonachlor	20
Methoxychlor	20
Endosulfan I	20
Endosulfan II	20
Endosulfan sulfate	20
Hexachlorobenzene	20
Dibutyl chlorendate	20
PCB-155 (std)	20
PCB-65 (std)	20

PCB	Conc. (ppb)	PCB	Conc. (ppb)	PCB	Conc. (ppb)	PCB	Conc. (ppb)
4+10	20	**45**	5	**92+84**	10	**163+138**	10
7+9	20	**52**	5	**89**	5	**126**	5
6	10	**49**	5	**101**	5	**166**	5
8+5	20	**47**	5	**99**	5	**128**	5
14	20	**48**	5	***trans*-Nonachlor**	5	**167**	5
19	5	**65**	5	**119**	5	**174**	5
30	8	**44**	5	**83**	5	**202+171**	10
12	10	**37**	5	**97**	5	**156**	5
13	10	**42**	5	**81**	5	**204**	6
18	5	**41+71**	10	**87**	5	**172**	5
15+17	15	**64**	5	**85**	5	**180**	5
16	5	**100**	5	**77**	5	**199**	5
32	5	**Octachlorostyrene**	5	**110**	5	**169**	5
26	5	**74**	5	**135+144**	10	**170+190**	10
31	5	**70+76**	10	**123+149**	10	**201**	5
28	5	**66**	5	**118**	5	**207**	5
33	5	**95**	5	**114**	5	**194**	5
53	5	**91**	5	**131**	5	**205**	5
22	5	**56+60**	10	**132+153+105**	15	**206**	5

Figure 1. List of compounds present in each standard with the concentration of each analyte (in ppb). Standards contained either PAHs, pesticides, or PCBs.

Experimental

Samples were collected from both air and precipitation using XAD-2, then fractionated into hexane and a 1:1 mix of hexane and DCM. An Agilent 8890 GC with Agilent 7000E QQQ was used for this analysis. Injections were pulsed in splitless mode on an MMI inlet with a bottom-fritted liner. The final method contained 425 MRMs across 181 MRM groups. Standards were prepared in hexane for all 140 compounds.

Table 1. Agilent 8890 GC Parameters

GC and MS Conditions:	
Injection vol.	1µL
Inlet	MMI @ 280°C pulsed splitless mode 25 psi pulse until 0.5 min Purge 30mL/min at 0.75 min
Column	HP-5MS Column (30m x 250µm, 0.25mm)
Carrier gas	1.0 mL/min constant flow (helium)
Oven	60°C hold 1.0 min Ramp 40°C/min → 170°C, no hold Ramp 10°C/min → 310°C, hold 3 min
MSD Transfer Line	320°C

Figure 2. Agilent 8890 GC with Agilent 7000E QQQ used for this analysis.

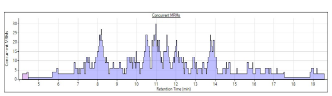

Figure 3. Plot of MRMs set in the acquisition method over time. As many as 30 MRMs occur simultaneously.

Results and Discussion

Standards were run to develop a dMRM method.

Standards were prepared for each of the three compound classes (pesticide, PAH, and PCB) and retention times were assigned by running in scan mode and analyzing the data in MassHunter Unknowns Analysis. The Pesticide and Environmental Pollutant (P&EP) Database assisted with identifying the elution order of the compounds. MRM transitions were developed for all compounds of interest to analyze on GC-QQQ by using a combination of the P&EP Database, literature, and Optimizer software. Samples were run in dynamic MRM (dMRM) mode with at least 1 quantifier ion and 1 qualifier ion transition per analyte.

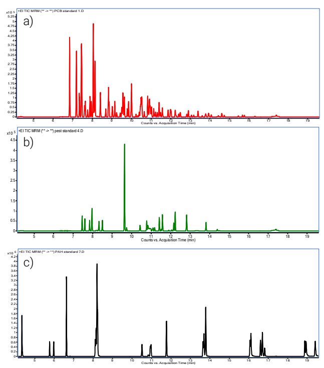

Figure 4. Total ion chromatograms of standards for a) PCBs, b) pesticides, and c) PAHs.

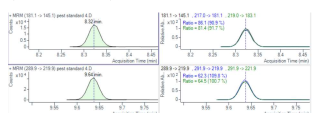

Figure 5. Example extracted ion chromatograms of quantifier and qualifier transitions for the pesticide δ-BHC.

Sample quantification by GC/MS/MS is comparable to GC-ECD analyses.

Samples containing various mixtures of pesticides, PAHs, and PCBs were analyzed by this GC/MS/MS method, after previously being analyzed by GC-ECD. Quantification of all compounds by GC/MS/MS is comparable to historical data by GC-ECD. Using a single unified acquisition method, 24 PAHs, 24 pesticides, and 92 PCBs have been identified from environmental samples at low ppb levels. Final concentrations of the samples ranged from 3 to 675 ppb.

Table 2. List of samples that were quantified with analyte class of interest, original sample matrix, and fraction.

Sample Number	Analyte Class of Interest	Original Sample Matrix	Current Solvent	IUB Concentration	IUB Batch	IUB Fraction
1	Polychlorinated biphenyls	Air/Vapor Phase (XAD)	Hexane	500 ng/ul	JU22C	Hexane
	Pesticides	Air/Vapor Phase (XAD)	Hexane	70 ng/ul	JU22C	Hexane:DCM
2	Pesticides	Precipitation (XAD)	Hexane	15 ng/ul	M22P	Hexane:DCM
3	Polychlorinated biphenyls	Precipitation (XAD)	Hexane	35 ng/ul	JL22P	Hexane
4	Polychlorinated biphenyls	Matrix Spike (XAD)	Hexane	675 ng/ul	F2M22C	Hexane
	Pesticides	Matrix Spike (XAD)	Hexane	300 ng/ul	F2M22C	Hexane:DCM
5	Pesticides	Air/Particle Phase (Filter)	Hexane	5 ng/ul	J22F	Hexane:DCM
6	Polychlorinated biphenyls	Lab Blank Vapor (XAD)	Hexane	3 ng/ul	JL22C	Hexane
7	Pesticides	Air/Vapor Phase (XAD)	Hexane	35 ng/ul	M222C	Hexane:DCM
	Polychlorinated biphenyls	Air/Vapor Phase (XAD)	Hexane	13 ng/ul	M222C	Hexane

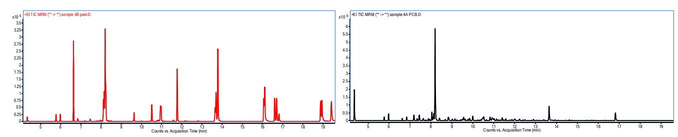

Figure 6. Total ion chromatograms for example pesticide (left) and PCB (right) samples that were quantified by GC/MS/MS.

Table 3. Quantification of pesticides in each sample (concentrations shown in ppb). Retention times and quantifier MRM transition are shown, along with absolute response.

			Sample 1B pest		Sample 2 pest		Sample 4B pest		Sample 7a pest		Sample 5 pest	
Name	Transition	RT	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.
Aldrin	254.9 -> 220.0	9.78					1148	3.49				
BHC-alpha (benzene hexachloride)	216.9 -> 181.0	7.47	544	0.369	1264	0.755	15782	10.75	46	0.019	2386	1.236
BHC-beta	181.0 -> 145.0	7.85	680	0.582	914	0.689	14507	12.47	934	0.488	582	0.38
BHC-delta	181.1 -> 145.1	8.32	2560	2.213	997	0.759	13521	11.75			6592	4.357
BHC-epsilon	180.9 -> 144.9	8.51	18368	11.257	20611	11.128	19628	12.09	9369	3.504	25562	11.975
BHC-gamma (Lindane, gamma HCH)	181.0 -> 145.0	7.97	555	0.436	1380	0.954	12849	10.14	194	0.093	225	0.135
Chlordane-cis	271.8 -> 236.9	11.18			2002	3.932	4334	9.62				
Chlordane-oxy	114.9 -> 51.1	10.44			1346	1.865	7108	11.13				
Chlordane-trans	271.7 -> 236.9	10.84	446	0.809	3545	5.674	6736	12.19	701	0.78	79	0.111
DDD-o,p'	235.0 -> 165.1	11.58					64971	15.70				
DDD-p,p'	237.0 -> 165.1	12.23			803	0.44	6605	4.09				
DDE-p,p'	246.1 -> 176.2	11.42			853	0.255	1929	0.65				
DDT-o,p'	235.0 -> 165.2	12.24			894	0.32	10138	4.11				
DDT-p,p'	235.0 -> 165.2	12.82			1805	0.65	19710	8.02				
Dibutyl chlorendate	237.0 -> 236.5	14.39	5700	17.041	3032	7.986	3634	10.82	7021	12.849	4423	10.245
Dieldrin	262.9 -> 193.0	11.51	970	3.616	4023	13.214	2959	10.99	377	0.861		
Endosulfan I (alpha isomer)	194.9 -> 160.0	11.06					1743	11.38				
Endosulfan II (beta isomer)	206.9 -> 172.0	12.06					1695	9.87				
Endosulfan sulfate	271.9 -> 237.0	12.81					7841	10.01				
Endrin	262.8 -> 193.0	11.91					2188	14.00				
Heptachlor exo-epoxide	352.8 -> 262.9	10.42			1517	4.384	3740	12.34				
Hexachlorobenzene	283.8 -> 213.9	7.6			50	0.031	65	0.05			102	0.055
Methoxychlor, p,p'-	227.0 -> 169.1	13.81					13119	10.90			754	0.488
Nonachlor, trans-	271.8 -> 236.9	11.18	16	0.038	2296	4.905	4372	10.56			101	0.19

Results and Discussion

Analysis time was shortened significantly by using GC/MS/MS.

The analysis time for 140 pesticides, PAHs, and PCBs has been sped up about 17X. With the original methodology, three injections were needed over multiple hours to quantify all 140 analytes. This initial analysis by GC-ECD took 165 minutes for PCBs, a separate 165 minutes for pesticides, and another 24 minutes for PAHs. Running each sample therefore took about 6 hours to look for all analytes of interest. This new QQQ method allows for one single injection that takes under 21 minutes. GC/MS/MS therefore creates a significant time savings and allows full calibrations to now be completed in hours instead of multiple days.

Conclusions

Transitioning methods from GC-ECD to GC/MS/MS is highly beneficial.

By running in MRM mode, we enable the following:

- Significant time-savings
- Increased confidence in hits
- Sample quantification remains the same

References

[1]Zohair, A., et al. "Residues of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides in organically-farmed vegetables." *Chemosphere* 63.4 (2006): 541-553.

Acknowledgements

We would like to thank Daniel Lehman, Staci Capozzi and Karen Arnold (Indiana University, Bloomington, Indiana) for preparing standards and samples.

DE93540765