Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Analyzing Multi-Class Persistent Organic Pollutants (OCPs, PCBs, PBDEs, and PAHs) in Food Matrices in a Single Injection by APGC-MS/MS

Marie-Eve Rousseau,[1] Serge Fortier,[1] Luc Gagnon,[1] Eimear McCall,[2] Dominic Roberts,[2] Jody Dunstan[2]
[1]Ministère de l'Agriculture, des Pêcheries et de l'Alimentation (MAPAQ), Quebec, Canada, [2]Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Increased sensitivity and selectivity for GC amenable POPs (OCPs, PAHs, PBDEs and PCBs).

- Improved response of quasi-molecular ion when compared with traditional EI spectra.

- Accurate and sensitive quantification of 141 POPs, including coronene and dibenzo pyrene compounds by APGC.

- Ability to analyze multi-class compounds in a single injection with generic sample preparation.

- The sensitive Xevo® TQ-S is suitable for quantification and confirmation of food and environmental contaminants, and readily interchangeable with UPLC® and APGC.

WATERS SOLUTIONS

Atmospheric Pressure Gas Chromatography (APGC)

Xevo® TQ-S

MassLynx® Software

KEY WORDS

Atmospheric pressure gas chromatography, APGC, organochlorine pesticides, OCPs, polycyclic aromatic hydrocarbons, PAHs, polychlorinated biphenyls, PCBs, polybrominated diphenyl ethers, PBDEs, persistent organic pollutants, POPs

INTRODUCTION

The application of gas chromatography coupled to mass spectrometry (GC-MS) is well established and documented for the analysis of ubiquitous environmental contaminants, such as persistent organic pollutants (POPs). Four classes of globally regulated POPs are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs).

Common physicochemical characteristics of these POPs include resistance to chemical and biological degradation and high lipophilicity, thus resulting in their persistence and significant potential to bioaccumulate. Many of these compounds are classified as toxic, carcinogenic, and probable carcinogenic. Such is the concern regarding these pollutants that international treaties, in association with regional legislation, requires continued monitoring to ensure human safety.

Due to the structural similarities of many POP congeners and the complexity of these compounds, their analysis can prove challenging. Traditional GC-MS and MS/MS techniques by electron impact (EI) ionization have been favored, due to the volatility, thermal stability, and non-polarity of the compound. However, given the hard ionization associated with EI, extensive fragmentation can impact the abundance of the molecular ion and compound specific spectra. Atmospheric pressure gas chromatography (APGC) allows for a softer ionization technique, thus providing a more abundant molecular ion. Operated at atmospheric pressure, compounds are ionized by corona discharge in the presence of nitrogen. This ionization reaction, depending on the analytes of interest, can occur by two processes: charge transfer (under dry conditions) and proton transfer (in the presence of a protic solvent).

In this application note, we describe the development and validation of a quantitative method for 141 multi-class POP compounds in a variety of foodstuffs to ensure continued monitoring and consumer safety in Quebec, Canada. The sensitivity, selectivity, and quantification capability of APGC, when coupled with the Waters® Xevo TQ-S will be determined, using a generic sample preparation method for the satisfactory extraction of all analytes from a variety of food matrices.

EXPERIMENTAL

GC conditions

GC system:	7890A
Injector:	Splitless
Injection:	1 µL
Temp.:	300 °C
Column:	DB-5 (J&W, USA) 30 m x I.D. 0.25 mm x df 0.25 µm
Guard column:	120 cm fused silica hi-temp
Interface:	55 cm fused silica hi-temp
Temp. gradient:	70 °C (hold for 1 min), 12 °C.min^{-1} to 250 °C, 5 °C.min^{-1} to 280 °C, 4 °C.min^{-1} to 310 °C (hold for 4.5 min)
Transfer line temp.:	340 °C
Carrier gas flow:	1.5 mL.min^{-1} (helium)
Auxiliary gas:	350 l.h^{-1} (nitrogen)
Make-up gas:	250 mL.min^{-1} (nitrogen)

MS conditions

MS system:	Xevo TQ-S
APCI corona pin current:	2.5 µA
Cone gas:	250 l.h^{-1} (nitrogen)
Acquisition mode:	multiple reaction monitoring (MRM) in positive ionization for all four classes of POPs shown in Table 3
Data management:	MassLynx Software with TargetLynx Application Manager

Sample preparation

Milk, infant formula, beef, pork, chicken, and fish were analyzed for PCB, PBDE, OCP, and PAH compounds using the following generic extraction procedure. Homogenized sample (12 g) was placed in a 50-mL glass centrifuge tube and fortified with internal standard, as described in Table 1. A smaller sample portion (10 g) was weighed for foods with high fat content (*e.g.* beef). Water (5 mL) was added to the solid food samples and reconstituted powder milk formulae. Samples were vortexed and allowed to stand for 20 minutes. Ethyl acetate (10 mL) was added and samples were shaken vigorously for 1 minute. QuEChERS salts, magnesium sulphate (4 g), and sodium chloride (2 g) were added to the tubes and shaken vigorously for an additional minute.

Following centrifugation, the supernatant (5 mL) was removed, evaporated, and reconstituted in dichloromethane (2.4 mL). It was then filtered through 0.45 µm PTFE filters in preparation for gel permeation chromatography (GPC). EnvirosepABC GPC pre column (60 x 21.2 mm) and column (350 x 21.2 mm) were used, with dichloromethane as eluent (5 mL.min^{-1}). The resultant extract was transferred to a suitable tube for evaporation, where the GPC collection tube was rinsed three times with dichloromethane. These rinses were combined with the original extract and evaporated to 750 µL. The volume was then made up to 1 mL in hexane and silica gel cleanup was performed. Silica columns were prepared by adding silica (2 g) into a 1-cm wide borosilicate glass column with a glass wool frit. These columns were conditioned with 3:1 hexane: dichloromethane solution (12 mL), followed by hexane (8 mL). The samples were loaded and eluted using 3:1 hexane: dichloromethane solution (20 mL). These extracts were evaporated to <0.5 mL, and fortified once more with the internal standard (25 µL, compounds marked with ** in Table 1). All samples were made up to 500 µL volume with isooctane, vortexed, and analyzed using the Xevo TQ-S with APGC.

Validation procedure

Method efficiency was determined and validated based on an in-house document that was inspired by several internationally recognized documents.[5-15] The limits of detection (LODs) and limits of quantification (LOQs) were determined using fortified replicates (n= 10) for all analytes in each matrix. This was carried out in accordance with the IUPAC method, *i.e.* LOD= 3 x std deviation of experimental noise, and LOQ= 10 x standard deviation of experimental noise.

The lowest limit (LL) was determined for all analytes where subsequent validation work and statistical analysis were based on this. Analyte recovery, repeatability (%RSD), and linearity were investigated. Replicate samples (n= 9) were prepared for each matrix (n= 6) at three fortification levels: 0.2 x LL, LL, and 2 x LL. From these replicates, the recovery and method repeatability were determined individually for each matrix.

Table 1. Internal standards for each class of POP, in order of retention time.
**Signifies internal standards added prior to injection.

	Compound	RT min	MRM m/z	Cone V	Collision eV	MRM m/z	Cone Voltage	Collision eV	Spiking level (µg.kg⁻¹)
1	Naphtalene d-8	6.38	136>108	55	20	136>84	55	20	0.5
2	2-methylnaphtalene-d10	7.65	152>150	40	18	152>122	40	28	0.5
3	Acenaphtylene-d8	9.44	160>158	65	28	160>156	65	30	0.5
4	Acenaphtene-d10	9.78	164>162	40	20	164>160	40	30	0.5
5	PCB #3 C-13	10.75	200>164	30	25	202>164	30	25	0.5
6	Fluorene-d10	10.78	176>174	35	20	176>172	35	35	0.5
7	Hexachlorobenzene C-13	12.11	294>222	30	35	294>257	30	30	0.5
8	BHC beta C-13	12.42	225>189	30	15	225>187	30	15	0.5
9	BHC gamma C-13	12.54	225>189	30	15	225>187	30	15	0.5
10	Phenanthrene-d10	12.69	188>186	65	25	188>160	65	40	0.5
11	PCB #15 C-13	12.75	234>164	30	25	236>164	30	25	0.5
12	Anthracene-d10	12.79	188>186	65	25	188>160	65	40	0.5
13	PCB #31 C-13	13.49	268>198	30	25	270>198	30	25	0.5
14	PCB #52 C-13	14.1	304>232	30	25	304>234	30	25	0.5
15	Heptachlore epoxyde cis C-13	15.05	363>262	30	20	363>292	30	20	0.5
16	Oxychlordane C-13	15.07	397>296	30	18	397>262	30	40	0.5
17	Fluoranthene-d10	15.1	212>210	70	35	212>208	70	35	0.5
18	Pyrene-d10	15.53	212>210	70	35	212>208	70	35	0.5
19	Nonachlor trans C-13	15.76	417>310	30	25	417>270	30	30	0.5
20	PCB #81 C-13	15.96	304>232	30	25	304>234	30	25	0.5
21	DDE p,p C-13	15.98	328>258	30	25	330>260	30	25	1.25
22	Dieldrine C-13	16.06	313>242	30	22	313>278	30	18	0.5
23	PCB #77 C-13	16.12	304>232	30	25	304>234	30	25	0.5
24	PCB #123 C-13	16.5	337.9>268	30	25	337.9>266	30	25	0.5
25	PCB #118 C-13	16.54	337.9>268	30	25	337.9>266	30	25	1
26	PBDE #28 C-13	16.56	417.8>258	35	25	417.8>260	35	25	0.5
27	PCB #114 C-13	16.73	337.9>268	30	25	337.9>266	30	25	0.5
28	DDT o,p C-13	16.74	247>211	30	20	249>213	30	20	0.5
29	PCB #153 C-13	16.91	371.8>301.9	30	30	371.8>299.9	30	30	0.5
30	PCB #105 C-13	16.99	337.9>268	30	25	337.9>266	30	25	0.5
31	DDT p,p C-13	17.32	247>211	30	20	249>213	30	20	0.5
32	PCB #138 C-13**	17.41	371.8>301.9	30	30	371.8>299.9	30	30	0.5
33	PCB #126 C-13	17.57	337.9>268	30	25	337.9>266	30	25	0.5
34	PCB #167 C-13	17.93	371.8>301.9	30	30	371.8>299.9	30	30	0.5
35	Benz[a]anthracene-d12	18.22	240>240	30	15	240>236	30	30	0.5
36	Chrysene-d12	18.31	240>240	30	15	240>236	30	30	0.5
37	PCB #156 C-13	18.38	371.8>301.9	30	30	371.8>299.9	30	30	0.5
38	PCB #157 C-13	18.5	371.8>301.9	30	30	371.8>299.9	30	30	0.5
39	PCB #180 C-13	18.71	405.8>335.9	30	30	405.8>333.9	30	30	0.5
40	PBDE #47 C-13	18.77	497.7>337.9	35	25	497.7>339.9	35	25	0.5
41	PCB #169 C-13	19.15	371.8>301.9	30	30	371.8>299.9	30	30	0.5
42	Mirex C-13	19.39	277>242	30	20	277>240	30	20	0.5
43	5-methylchrysene-d3**	19.59	245>244	45	25	245>242	45	40	0.5
44	PCB #189 C-13	19.95	405.8>335.9	30	30	405.8>333.9	30	30	0.5
45	PBDE #100 C-13	20.73	575.6>415.8	35	25	575.6>417.8	35	25	0.5
46	PCB #194 C-13	20.76	439.8>369.8	30	35	439.8>367.8	30	35	0.5
47	Benzo[b,k]fluoranthene-d12	21.3	264>264	30	15	264>260	30	30	1
48	PBDE #99 C-13	21.32	575.6>415.8	35	25	575.6>417.8	35	25	0.5
49	PCB #206 C-13	21.67	475.7>405.8	30	35	475.7>403.8	30	35	0.5
50	Benzo[e]pyrene-d12	21.99	264>264	30	15	264>260	30	30	0.5
51	Benzo[a]pyrene-d12	22.14	264>264	30	15	264>260	30	30	0.5
52	Perylene-d12	22.38	264>264	30	15	264>260	30	30	0.5
53	PCB #209 C-13	22.44	509.7>439.8	30	35	509.7>437.8	30	35	0.5
54	PBDE #154 C-13	23.15	655.5>495.7	35	25	655.5>493.7	35	25	1
55	PBDE #153 C-13	24.09	655.5>495.7	35	25	655.5>493.7	35	25	1
56	PBDE #138 C-13**	25.5	655.5>495.7	35	25	655.5>493.7	35	25	1
57	Indeno[1,2,3-cd]pyrene-d12	25.59	288>288	40	15	288>284	40	40	0.5
58	Dibenzo[a,h]anthracene-d14	25.68	292>292	40	15	292>288	40	30	0.5
59	Benzo[g,h,i]perylene-d12	26.34	288>288	40	15	288>284	40	40	0.5
60	PBDE #183 C-13	27.23	733.4>573.6	35	25	733.4>575.6	35	25	1
61	Coronene-d12	31.16	312>312	70	15	312>308	70	55	0.5
62	Dibenzo[a,e]pyrene 6C13	31.32	308>308	70	15	308>306	70	40	0.5

RESULTS AND DISCUSSION

The Xevo TQ-S with APGC was evaluated as an accurate and sensitive instrument for the detection of multi-class POP compounds (PAHs, PCBs, PBDEs, and OCPs). Over 140 analytes (excluding internal standards) were targeted in this method and represent the most common congener mixes and regulated POP compounds, covering low, medium, and high boiling compounds.

The accurate detection and quantification of certain compounds, including coronene, dibenzo pyrenes, and BDE #183 can often prove challenging during traditional GC-EI-MS analysis. Despite their complex structures and higher boiling points, these compounds are readily analyzed by APGC, where excellent LODs were achieved, as shown in Table 3 on pages 5-6.

A generic extraction method was developed for all analytes using the internal standards described in Table 1. Excellent recoveries, linearity, and LODs were determined for all analytes across the six different matrices. Comparable recoveries, with satisfactory repeatability, were achieved for the analytes in all matrices, as shown in the Table 2, using the recovery of PCB #126 as a representative example.

Table 2. Excellent recoveries achieved for PCB #126 from all matrices, showing robust efficiency of the generic sample cleanup procedure.

	Sample matrix	Recovery (%)	Repeatability (%RSD)
1.	Milk	104	3.1
2.	Infant formula	106	6.1
3.	Beef	103	5.9
4.	Chicken	103	6.7
5.	Pork	106	8.9
6.	Fish	109	2.8

The validated method using TQ-S with APGC was submitted and successfully accredited in accordance with international standard ISO 17025. For ease of discussion, the method's results for multi-class analytes will be demonstrated from here on using pork meat. The results shown in Table 3 focus on analyte recoveries and repeatability at the lowest fortification level in order to demonstrate system sensitivity and robustness at trace levels. Furthermore, the linearity and limits of detection, as summarized in Table 3 are discussed.

Table 3. MRM transitions for all POPs analyzed. Satisfactory recovery and repeatability results obtained for the four classes of POP compounds at the fortified levels shown in pork. Excellent limits of detection were achieved for all analytes.

	Compound	RT	MRM	Cone	Collision	MRM	Cone	Collision	Spiking level	Recovery	RSD	LOD
		min	m/z	V	eV	m/z	V	eV	(µg.kg⁻¹)	%	%	(µg.kg⁻¹)
1	Naphtalene	6.42	128>102	55	20	128>78	55	20	1	114	14.7	0.2
2	2-methylnaphtalene	7.72	142>141	40	18	142>115	40	28	1	97	10.9	0.2
3	Acenaphtylene	9.46	152>151	65	28	152>150	65	30	0.05	114	13.4	0.02
4	Acenaphtene	9.83	154>153	40	20	154>152	40	30	0.2	96	16.0	0.04
5	PCB IUPAC #1	9.96	188>152	30	25	190>152	30	25	0.005	97	3.6	0.0006
6	Pentachlorobenzene	10.22	250>215	30	25	250>179	30	30	0.05	97	3.1	0.005
7	PCB IUPAC #3	10.76	188>152	30	25	190>152	30	25	0.005	96	7.9	0.001
8	Fluorene	10.84	166>165	35	20	166>164	35	35	0.2	92	9.6	0.05
9	PCB IUPAC #10 (#4)	11.17	222>152	30	25	224>152	30	25	0.01	99	4.4	0.001
10	PCB IUPAC #8	11.94	222>152	30	25	224>152	30	25	0.005	113	4.8	0.0009
11	BHC alpha	11.96	217>181	30	15	219>183	30	15	0.05	100	5.0	0.008
12	Hexachlorobenzene	12.11	284>214	30	35	284>249	30	30	0.05	103	10.4	0.02
13	PCB IUPAC #19	12.32	256>186	30	25	258>186	30	25	0.005	103	4.2	0.0006
14	BHC beta	12.42	217>181	30	15	219>183	30	15	0.05	96	8.2	0.01
15	BHC gamma	12.55	217>181	30	15	219>183	30	15	0.05	94	6.6	0.009
16	PCB IUPAC #18 (#17)	12.71	256>186	30	25	258>186	30	25	0.005	116	7.9	0.001
17	Phenanthrene	12.74	178>177	65	25	178>151	65	40	0.2	91	17.0	0.04
18	PCB IUPAC #15	12.75	222>152	30	25	224>152	30	25	0.005	97	5.6	0.0009
19	Anthracene	12.83	178>177	65	25	178>151	65	40	0.05	93	12.4	0.007
20	HCH delta	12.95	217>181	30	15	219>183	30	15	0.05	99	4.1	0.006
21	PCB IUPAC #54	13.29	292>220	30	25	292>222	30	25	0.005	108	6.5	0.001
22	PCB IUPAC #28 (#31)	13.52	256>186	30	25	258>186	30	25	0.05	122	4.7	0.001
23	PCB IUPAC #33	13.69	256>186	30	25	258>186	30	25	0.005	97	4.5	0.0006
24	Heptachlore	13.81	337>266	30	20	337>302	30	15	0.05	105	6.5	0.009
25	Kepone	13.81	272>237	30	20	272>235	30	20	0.05	111	3.0	0.005
26	PCB IUPAC #22	13.82	256>186	30	25	258>186	30	25	0.005	100	4.5	0.0006
27	PCB IUPAC #52	14.1	292>220	30	25	292>222	30	25	0.05	118	4.8	0.004
28	PCB IUPAC #49 (#47)	14.18	292>220	30	25	292>222	30	25	0.005	118	10.0	0.002
29	PCB IUPAC #104	14.39	325.9>256	30	25	325.9>254	30	25	0.005	112	4.3	0.0006
30	Aldrin	14.41	293>186	30	40	293>220	30	25	0.05	92	6.6	0.009
31	PCB IUPAC #44	14.45	292>220	30	25	292>222	30	25	0.05	113	2.9	0.002
32	PCB IUPAC #37	14.52	256>186	30	25	258>186	30	25	0.005	105	7.5	0.001
33	PCB IUPAC #41	14.67	292>220	30	25	292>222	30	25	0.005	118	6.2	0.001
34	PCB IUPAC #40	14.8	292>220	30	25	292>222	30	25	0.005	106	6.6	0.001
35	PCB IUPAC #74	15.04	292>220	30	25	292>222	30	25	0.05	112	3.1	0.002
36	Heptachlore epoxyde cis	15.05	353>253	30	20	353>282	30	20	0.05	100	5.8	0.008
37	Oxychlordane	15.08	387>287	30	18	387>253	30	40	0.05	103	6.0	0.009
38	PCB IUPAC #70	15.09	292>220	30	25	292>222	30	25	0.05	110	5.5	0.003
39	Fluoranthene	15.13	202>201	70	35	202>200	70	35	0.2	106	3.4	0.02
40	Heptachlore epoxyde trans	15.13	353>253	30	20	353>282	30	20	0.05	101	5.2	0.01
41	PCB IUPAC #66	15.16	292>220	30	25	292>222	30	25	0.05	112	3.9	0.002
42	PCB IUPAC #95	15.17	325.9>256	30	25	325.9>254	30	25	0.05	114	8.1	0.003
43	PCB IUPAC #155	15.39	359.8>289.9	30	30	359.8>287.9	30	30	0.005	94	5.9	0.0009
44	PCB IUPAC #60	15.44	292>220	30	25	292>222	30	25	0.05	120	6.0	0.002
45	Chlordane cis	15.44	373>266	30	25	373>301	30	20	0.05	103	4.1	0.007
46	DDE o,p	15.49	316>246	30	25	318>248	30	25	0.05	101	2.4	0.004
47	PCB IUPAC #101 (#90)	15.53	325.9>256	30	25	325.9>254	30	25	0.1	114	6.0	0.007
48	Pyrene	15.56	202>201	70	35	202>200	70	35	0.2	98	10.7	0.02
49	PCB IUPAC #99	15.62	325.9>256	30	25	325.9>254	30	25	0.005	108	13.3	0.002
50	Endosulfane I	15.65	339>160	30	20	339>196	30	20	0.05	102	7.7	0.01
51	Chlordane trans	15.69	373>266	30	25	373>301	30	20	0.05	103	4.2	0.007
52	PCB IUPAC #119	15.72	325.9>256	30	25	325.9>254	30	25	0.005	99	8.5	0.001
53	Nonachlor trans	15.77	407>300	30	25	407>263	30	30	0.05	104	7.0	0.01
54	PCB IUPAC #87	15.96	325.9>256	30	25	325.9>254	30	25	0.05	118	4.6	0.003
55	PCB IUPAC #81	15.97	292>220	30	25	292>222	30	25	0.005	107	7.6	0.002
56	DDE p,p	15.98	316>246	30	25	318>248	30	25	0.05	113	11.7	0.02
57	Dieldrine	16.07	303>232	30	22	303>268	30	18	0.05	122	5.6	0.01
58	PCB IUPAC #77	16.12	292>220	30	25	292>222	30	25	0.05	111	3.0	0.0006
59	PCB IUPAC #110	16.12	325.9>256	30	25	325.9>254	30	25	0.05	120	3.9	0.003
60	DDD o,p	16.13	235>199	30	20	237>201	30	20	0.05	90	4.7	0.006
61	PBDE #17 C-12	16.29	405.8>246	35	25	405.8>248	35	25	0.005	100	6.9	0.0006
62	PCB IUPAC #151	16.32	359.8>289.9	30	30	359.8>287.9	30	30	0.05	101	5.8	0.003
63	Endrin	16.43	380>345	30	12	380>279	30	20	0.05	116	7.2	0.01
64	PCB IUPAC #123	16.5	325.9>256	30	25	325.9>254	30	25	0.005	100	8.3	0.001
65	PCB IUPAC #149	16.51	359.8>289.9	30	30	359.8>287.9	30	30	0.05	103	9.0	0.003
66	PCB IUPAC #118	16.54	325.9>256	30	25	325.9>254	30	25	0.05	117	3.1	0.002
67	PBDE #28 C-12	16.56	405.8>246	35	25	405.8>248	35	25	0.005	106	4.9	0.0009
68	Endosulfane II	16.57	339>160	30	20	339>196	30	20	0.05	90	10.9	0.02
69	DDD p,p	16.66	235>199	30	20	237>201	30	20	0.05	92	5.4	0.008
70	PCB IUPAC #114	16.74	325.9>256	30	25	325.9>254	30	25	0.005	108	5.8	0.0009
71	DDT o,p	16.74	235>199	30	20	237>201	30	20	0.05	99	4.5	0.007
72	Nonachlor cis	16.79	407>300	30	25	407>263	30	30	0.05	105	4.2	0.0066
73	PCB IUPAC #188	16.82	393.8>323.9	30	30	395.8>325.9	30	30	0.005	106	5.4	0.0009
74	PCB IUPAC #153	16.92	359.8>289.9	30	30	359.8>287.9	30	30	0.05	114	6.6	0.004
75	PCB IUPAC #168 (#132)	16.97	359.8>289.9	30	30	359.8>287.9	30	30	0.005	106	18.8	0.003

#	Compound	RT min	MRM m/z	Cone V	Collision eV	MRM m/z	Cone V	Collision eV	Spiking level (µg.kg⁻¹)	Recovery %	RSD %	LOD (µg.kg⁻¹)
76	PCB IUPAC #105	17	325.9>256	30	25	325.9>254	30	25	0.005	106	10.8	0.002
77	PCB IUPAC #141	17.15	359.8>289.9	30	30	359.8>287.9	30	30	0.05	105	2.9	0.002
78	PCB IUPAC #137	17.27	359.8>289.9	30	30	359.8>287.9	30	30	0.005	98	7.0	0.0009
79	DDT p,p	17.33	235>199	30	20	237>201	30	20	0.05	103	14.6	0.02
80	PCB IUPAC #138	17.42	359.8>289.9	30	30	359.8>287.9	30	30	0.05	112	3.9	0.003
81	PCB IUPAC #158	17.47	359.8>289.9	30	30	359.8>287.9	30	30	0.005	97	4.6	0.0006
82	PCB IUPAC #129	17.57	359.8>289.9	30	30	359.8>287.9	30	30	0.005	108	4.9	0.0009
83	PCB IUPAC #178	17.57	393.8>323.9	30	30	395.8>325.9	30	30	0.005	105	9.1	0.001
84	PCB IUPAC #126	17.58	325.9>256	30	25	325.9>254	30	25	0.005	106	8.9	0.001
85	PCB IUPAC #187	17.72	393.8>323.9	30	30	395.8>325.9	30	30	0.05	111	5.0	0.003
86	Benzo[c]phenanthrene	17.77	228>228	30	15	228>226	30	30	0.05	101	6.1	0.004
87	PCB IUPAC #183	17.82	393.8>323.9	30	30	395.8>325.9	30	30	0.005	104	14.3	0.002
88	PCB IUPAC #167 (#128)	17.94	359.8>289.9	30	30	359.8>287.9	30	30	0.01	107	3.6	0.001
89	Cyclopenta[cd]pyrene	18.25	226>225	60	40	226>224	60	45	0.05	81	6.2	0.007
90	Benz[a]anthracene	18.27	228>228	30	15	228>226	30	30	0.05	107	8.7	0.005
91	PCB IUPAC #177	18.29	393.8>323.9	30	30	395.8>325.9	30	30	0.005	104	10.1	0.002
92	PCB IUPAC #202	18.36	427.8>357.8	30	30	427.8>355.8	30	30	0.005	101	5.9	0.0009
93	PBDE #49 C-12	18.36	485.7>325.9	35	25	485.7>327.9	35	25	0.005	105	5.2	0.0009
94	Chrysene	18.37	228>228	30	15	228>226	30	30	0.05	107	16.7	0.01
95	PCB IUPAC #171	18.38	393.8>323.9	30	30	395.8>325.9	30	30	0.005	101	4.3	0.0006
96	PCB IUPAC #156	18.39	359.8>289.9	30	30	359.8>287.9	30	30	0.005	106	6.6	0.001
97	PCB IUPAC #157	18.5	359.8>289.9	30	30	359.8>287.9	30	30	0.005	108	7.1	0.001
98	PCB IUPAC #201	18.52	427.8>357.8	30	30	427.8>355.8	30	30	0.005	103	6.0	0.0009
99	PCB IUPAC #180	18.72	393.8>323.9	30	30	395.8>325.9	30	30	0.05	113	4.4	0.003
100	PCB IUPAC #193	18.77	393.8>323.9	30	30	395.8>325.9	30	30	0.05	115	4.8	0.0009
101	PBDE #47 C-12	18.78	485.7>325.9	35	25	485.7>327.9	35	25	0.05	108	3.7	0.003
102	PCB IUPAC #191	18.86	393.8>323.9	30	30	395.8>325.9	30	30	0.005	96	6.7	0.0009
103	PCB IUPAC #200	19	427.8>357.8	30	30	427.8>355.8	30	30	0.005	104	6.8	0.001
104	PBDE #66 C-12	19.11	485.7>325.9	35	25	485.7>327.9	35	25	0.005	98	7.3	0.001
105	PCB IUPAC #169	19.15	359.8>289.9	30	30	359.8>287.9	30	30	0.005	102	6.8	0.0009
106	PCB IUPAC #170	19.34	393.8>323.9	30	30	395.8>325.9	30	30	0.005	99	9.2	0.002
107	Mirex	19.4	272>237	30	20	272>235	30	20	0.05	109	2.1	0.003
108	PCB IUPAC #199	19.53	427.8>357.8	30	30	427.8>355.8	30	30	0.005	97	5.9	0.0009
109	5-methylchrysene	19.62	242>241	45	25	242>239	45	40	0.05	99	4.8	0.005
110	PCB IUPAC #203	19.64	427.8>357.8	30	30	427.8>355.8	30	30	0.005	95	9.9	0.001
111	PBDE #77 C-12	19.67	485.7>325.9	35	25	485.7>327.9	35	25	0.005	95	12.7	0.0003
112	PCB IUPAC #189	19.96	393.8>323.9	30	30	395.8>325.9	30	30	0.005	101	7.3	0.001
113	PCB IUPAC #208	20.29	463.7>393.8	30	35	463.7>391.8	30	35	0.005	106	3.8	0.0006
114	PCB IUPAC #195	20.32	427.8>357.8	30	30	427.8>355.8	30	30	0.005	100	3.9	0.0006
115	PBDE #100 C-12	20.74	563.6>403.8	35	30	563.6>405.8	35	30	0.005	109	17.0	0.003
116	PCB IUPAC #194	20.77	427.8>357.8	30	30	427.8>355.8	30	30	0.005	111	6.3	0.001
117	PCB IUPAC #205	20.9	427.8>357.8	30	30	427.8>355.8	30	30	0.005	96	5.2	0.0009
118	PBDE #119 C-12	20.94	563.6>403.8	35	30	563.6>405.8	35	30	0.005	101	5.6	0.0006
119	PBDE #99 C-12	21.33	563.6>403.8	35	30	563.6>405.8	35	30	0.05	107	2.9	0.004
120	Benzo[b,j,k]fluoranthene	21.34	252>252	30	15	252>250	30	30	0.15	98	8.1	0.01
121	7,12-Dimethylbenzo[a]anthracene	21.38	256>241	45	25	256>239	45	40	0.05	100	3.8	0.007
122	PCB IUPAC #206	21.68	463.7>393.8	30	35	463.7>391.8	30	35	0.005	103	4.4	0.0006
123	Benzo[e]pyrene	22.06	252>252	30	15	252>250	30	30	0.05	93	11.9	0.004
124	Benzo[a]pyrene	22.2	252>252	30	15	252>250	30	30	0.05	93	12.9	0.005
125	PCB IUPAC #209	22.45	497.7>427.8	30	35	497.7>425.8	30	35	0.005	105	5.3	0.0009
126	Perylene	22.45	252>252	30	15	252>250	30	30	0.05	100	6.4	0.007
127	PBDE #85 C-12	22.51	563.6>403.8	35	30	563.6>405.8	35	30	0.005	100	5.0	0.0009
128	PBDE #154 C-12	23.16	643.5>483.7	35	25	643.5>481.7	35	25	0.01	101	6.4	0.002
129	3-Methylcholanthrene	23.44	268>252	50	40	268>253	50	30	0.05	105	11.6	0.006
130	PBDE #153 C-12	24.1	643.5>483.7	35	25	643.5>481.7	35	25	0.01	102	8.0	0.002
131	PBDE #138 C-12	25.51	643.5>483.7	35	25	643.5>481.7	35	25	0.01	100	5.7	0.002
132	Indeno[1,2,3-cd]pyrene	25.66	276>276	40	15	276>274	40	40	0.05	96	10.6	0.005
133	Dibenzo[a,h]anthracene	25.79	278>278	40	15	278>276	40	30	0.05	94	3.2	0.005
134	Benzo[g,h,i]perylene	26.42	276>276	40	15	276>274	40	40	0.05	103	13.7	0.007
135	Anthanthrene	26.83	276>276	40	15	276>274	40	40	0.05	66	8.3	0.006
136	PBDE #183 C-12	27.25	721.4>561.6	35	35	721.4>563.6	35	35	0.01	108	8.7	0.003
137	Dibenzo[a,l]pyrene	30.14	302>302	70	15	302>300	70	40	0.05	91	5.4	0.008
138	Coronene	31.25	300>300	70	15	300>298	70	55	0.05	104	4.9	0.01
139	Dibenzo[a,e]pyrene	31.33	302>302	70	15	302>300	70	40	0.05	83	5.7	0.007
140	Dibenzo[a,i]pyrene	31.79	302>302	70	15	302>300	70	40	0.05	95	5.2	0.007
141	Dibenzo[a,h]pyrene	32.05	302>302	70	15	302>300	70	40	0.05	86	12.6	0.005

Sensitivity and selectivity

Given the softer ionization associated with APGC, more abundant molecular ions can be observed compared with traditional EI spectra. During method development stages, all of the analytes showed better sensitivity for the molecular ion by charge transfer (in dry source) in comparison with protonation, given the electronegativity associated with chlorinated and brominated compounds.

The increased sensitivity observed for many POP analytes, in comparison with traditional GC-EI-MS methods can be seen in Figure 1. Here the signal-to-noise ratio (S/N) determined for BDE #17 and #28 shows a significant increase when analyzed by APGC. This further reduces matrix loading, thus improving liner, column life, and instrument robustness, while reducing instrument maintenance.
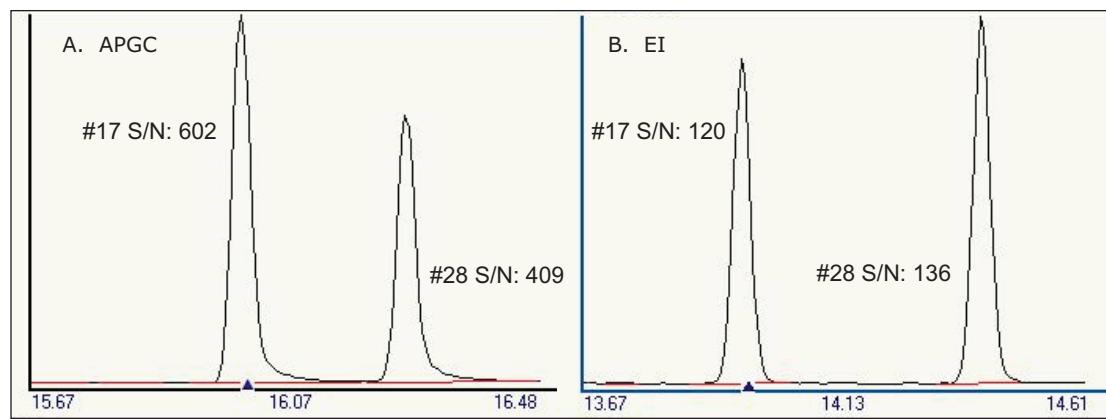
Figure 1. Increased sensitivity achieved for BDE #17 and #28 in extracted pork by A. APGC (2 pg on column) compared with traditional B. EI (20 pg on column).

The medium and high concentration replicates were combined (n= 18) to allow for an averaged and more representative statistical analysis of the method recovery and repeatability for each matrix. Further statistical analysis was completed to allow for validation of a robust method at low concentration levels. To this end, the method recovery and repeatability were determined separately for all analytes in each matrix at the lowest level of fortification (0.2 x LL, where n= 9). These results are shown for pork matrix in Table 3.

Limits of detection

The detection of POPs in food and environmental samples are challenging, because of their ubiquitous presence and the increasingly low detection levels required to meet regulatory limits in complex matrices. However, the analysis of PBDEs, PCBs, PAHs, and OCPs can be achieved below all the required levels of detection using APGC-MS.

The limits determined for all of the analytes are summarized in Table 3, where concentrations of <1 µg.kg⁻¹ were achieved for all analytes in pork extract. The excellent sensitivity achieved is further demonstrated in Figure 2, where the S/N ratio was determined for 50 fg on column. An example of each class of POP analyzed was compared to its matrix blank, thus demonstrating the selectivity afforded.
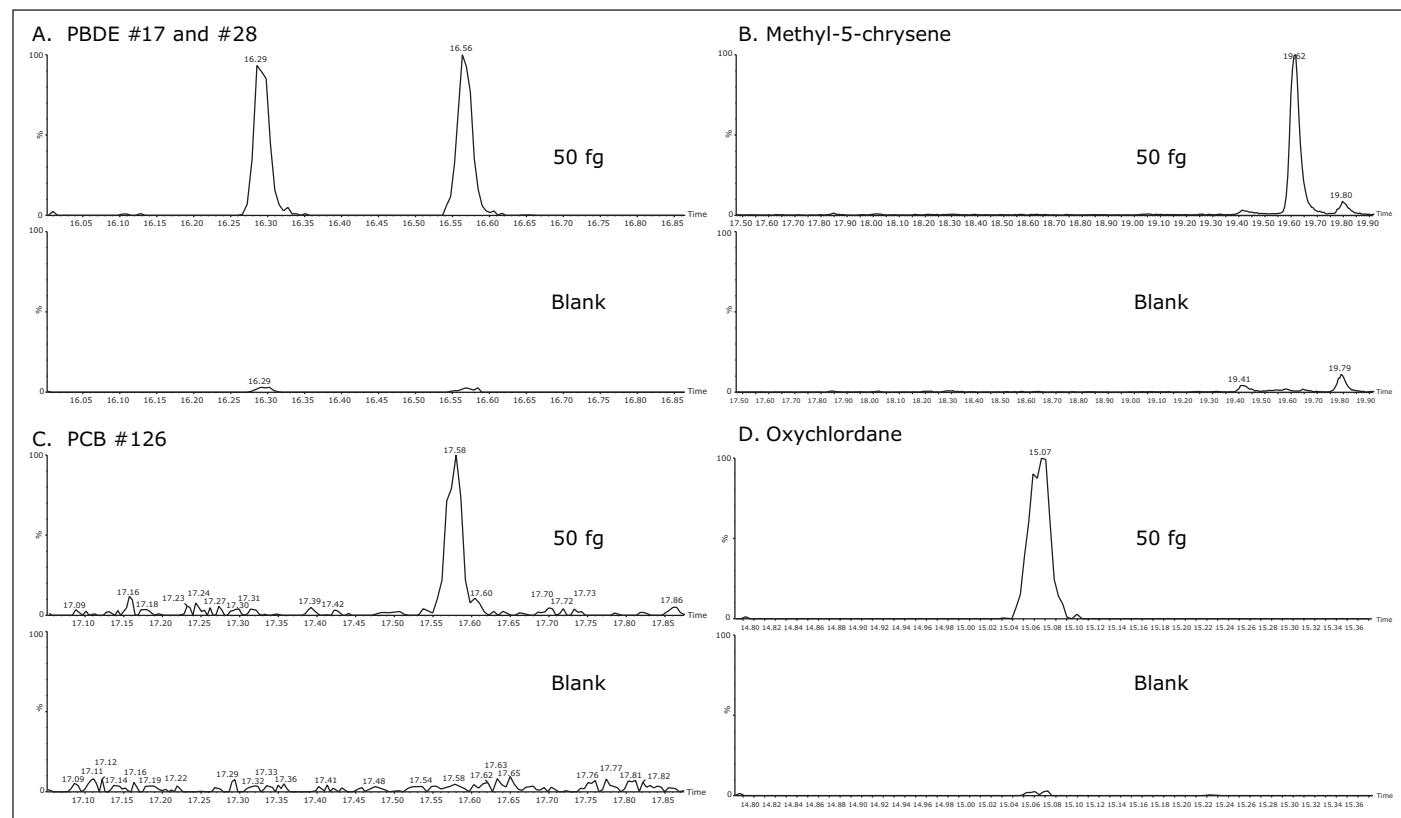
Figure 2. Excellent sensitivity and selectivity determined for 50 fg on column for: A. PBDE #17 and #28; B. methyl-5-chrysene; C. PCB #126; and D. oxychlordane, in comparison to a blank extracted pork sample.

The developed method allowed for excellent repeatability for the multi-class components fortified at low levels in a variety of matrices. This is well demonstrated by the validation data shown in Table 3, where excellent recoveries and method repeatability are shown for all analytes fortified in pork meat (n= 9) at levels between 50 to 1000 ng.kg^{-1}.

Using the optimized generic sample preparation and cleanup method, the percentage recoveries ranged from 65% to 122% in pork matrix. Percentage relative standard deviations (%RSD) were found to be <20% for all analytes. This is an acceptable level for multi-residue analysis in complex matrices, showing low variance for all of the PBDEs, PCBs, PAHs, and OCPs when spiked at parts per trillion (ppt, equating to ng.kg^{-1}) levels in the complex matrix.

Linearity

Linearity was investigated for all analytes utilizing the internal standards described in Table 1. Good correlation was achieved (R^2>0.99) over a satisfactory working range of 2 to 25 µg.kg^{-1}. This working range was deemed most appropriate, allowing for the accurate quantification for all analytes at legislated levels where applicable. An example of the calibration curves achieved utilizing the internal standard is provided in Figure 3 for each class of POP in pork meat.
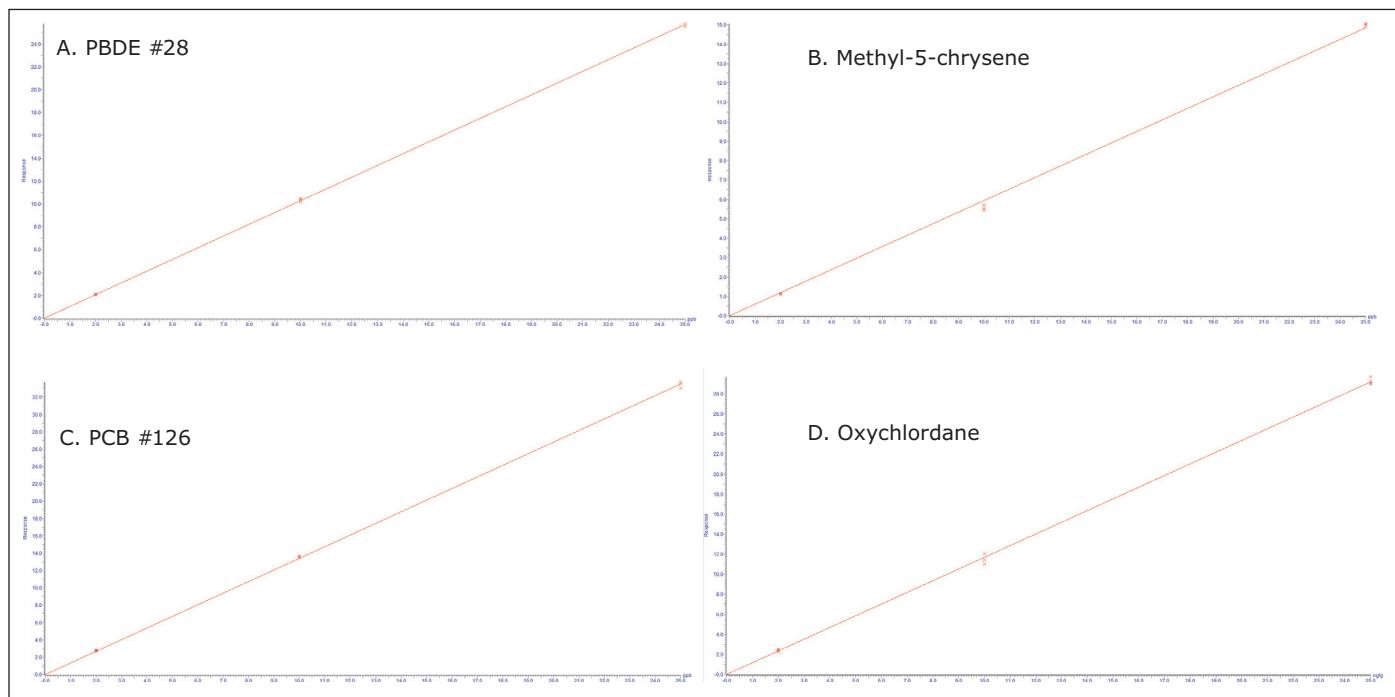
Figure 3. Example of excellent calibration curve correlation for each of the four POP classes over the concentration range 2 to 25 µg.kg^{-1} in pork meat for:
A. polybrominated diphenyl ethers; B. polycyclic aromatic hydrocarbons; C. polychlorinated biphenyls; D. organochlorine pesticides.

CONCLUSIONS

Time-consuming and costly analyses are a major drain on food and environmental testing laboratories, where multi-analyte methods are preferred for efficient use of resources. While the robust Xevo TQ-S can be coupled with UPLC to provide sensitive analysis of LC-amenable compounds, this work shows the ability of the Xevo TQ-S to analyze multi class POPs by atmospheric pressure gas chromatography with excellent robust sensitivity.

The optimization of a single cleanup method for a variety of analytes has been shown to achieve satisfactory recoveries, while allowing the Xevo TQ-S with APGC to quantify analytes below the regulatory limit. Taking pork as an example, excellent recoveries, in the range of 66% to 122% were determined, where the repeatability was <20% for all 141 POP analytes.

This validated and accredited method has been implemented by the MAPAQ for the routine analysis of a multitude of meats, fish, milk, and infant formula to ensure consumer safety in Quebec, Canada. When compared with traditional GC-EI-MS methods, increased sensitivity, less maintenance, and routine cleaning has been required for the Xevo TQ-S with APGC, further improving laboratory efficiency.

Acknowledgements

References

1. M van den Berg, L S Birnbaum, M Denison, M De Vito, W Farland, M Feeley, H Fiedler, H Hakansson, A Hanberg, L Haws, M Rose, S Safe, D Schrenk, C Tohyama, A Tritscher, J Tuomisto, M Tysklind, N Walker, R E Peterson. The 2005 World Health Organisation Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. *Tox. Sci.* 93 (2), 2006.

2. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals on Humans, *World Health Organisation/International Agency for Research on Cancer.* Volumes 1-42, 1979.

3. C Vanden Bilcke. The Stockholm Convention on Persistent Organic Pollutants. *Eur Comm Environ Law.* 11, 2002.

4. Food and Drugs Act (RSC) 1985, c. F-27, available at: http://laws-lois.justice. gc.ca/eng/acts/F-27/FullText.html. Last accessed 14 April 2014.

5. Guide pour la validation des méthodes d'essai physico-chimique et l'évaluation de l'incertitude de mesure, *Manuel suisse des données alimentaires.* 60C, 2004.

6. Guide pour la qualité en chimie analytique. *CITAC/EURACHEM.* 2002.

7. The fitness for purpose of analytical methods, A laboratory guide to method validation and related topics, *EURACHEM.* 1998.

8. Protocole d'évaluation d'une méthode alternative d'analyse quantitative par rapport à une méthode de référence. *AFNOR V03B.* 1993.

9. Guidelines for single-laboratory validation of analytical methods for trace-level concentrations for organic chemicals. *AOAC/FAO/IAEA and IUPAC.* 1998.

10. Implementing Council Directive 96/23EC concerning the performance of analytical methods and the interpretation of results. *CONSLEG 2002D0657.* 2004.

11. G T Wernivont. Use of statistics to develop and evaluate analytical methods. *AOAC.* 1985.

12. A Fajgelj, A Ambrus. Principles and practices of method validation. *The Royal Society of Chemistry.* 2000, DOI:10.1039/9781847551757-00001

13. Guidelines for evaluating acceptable methods of analyses, Codex committee on methods of analyses and sampling, *CX/MAS/02/4.* 2002.

14. Document de travail sur les méthodes d'analyse pour les résidus de médicaments vétériniares dans les aliments. *CX/RVDF/19/6.* 2010.

15. Exactitude (justesse et fidélité) des résultats et méthodes de mesure Partie 2: Méthode de base pour la détermination de la répétabilité et de la reproductibilité d'une méthode de mesure normalisée. *Organisation Internationale de Normalisation.* ISO 5725-2, 1994.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com