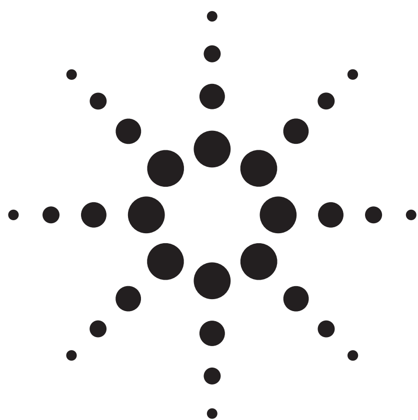

GC/µECD Analysis and Confirmation of PCBs in Fish Tissue with Agilent J&W DB-35ms and DB-XLB GC Columns

Application Note

Food Analysis

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Abstract

A fish sample obtained from a local grocery store was analyzed for 19 PCB (Polychlorinated Biphenyl) compounds using a GC with µECD. A QuEChERS procedure (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) cleaned the sample prior to analysis. A dual µECD and dual capillary GC column approach performed simultaneous primary and confirmatory analysis. The primary column, Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 µm and confirmatory column Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm effectively resolved all 19 PCBs. The method was calibrated at the 10, 20, 50, 100, 250, and 400 ng/mL PCB levels, yielding excellent linearity and reproducibility. Spiked recoveries ranged between 72 and 116% at 50 and 200 ng/mL levels in the fish matrix.

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Introduction

Omega-3 fatty acids lower cholesterol, cancer risks, and blood pressure levels. The human body does not produce this fatty acid that is mainly obtained through diet or supplements. Fish is a good source of omega-3 fatty acids, which is especially high in fatty fish. However, even though fish has many beneficial aspects, fish may also contain contaminants such as heavy metals, PCBs or other pollutants.

Even though polychlorinated biphenyls (PCBs) were banned in the late 1970s in the United States and other countries, PCB contamination remains a concern. PCBs are slow to break down and are persistent in the environment, collecting in the sediment of rivers and lakes [1,2]. PCBs are highly lipophilic and bioaccumulate in the fatty tissue of fish throughout the food chain. Consumption of contaminated fish is a significant source of human exposure [3]. Many agencies such as the EPA and local state governments have issued fish advisories recommending monthly or annual fish consumption limits [4].

A dual column, dual µECD system with an Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 µm primary analysis column and an Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm confirmatory column is shown to separate and analyze the PCBs in the fish sample. Continuous improvements and stringent process control with respect to column activity make this column pair a particularly good choice for analysis of PCBs in a challenging fish tissue matrix.

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) AOAC sample preparation approach is used for extraction and cleanup of 19 PCB congeners in fish tissue. This approach involves an initial extraction in a buffered aqueous and acetonitrile system, an extraction and partitioning step following a salt addition, and a cleanup step using dispersive solid phase extraction [5].

Experimental

An Agilent 7890A GC system equipped with dual µECD detection was used for this series of experiments enabling simultaneous identification and confirmation from a single injection. The GC was also fitted with an unpurged two-way splitter capillary flow technology (CFT) device, simplifying maintenance and reducing system downtime. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC:	Agilent 7890A equipped with dual µECD detection
Sampler:	Agilent 7873B 5.0 µL syringe (Agilent p/n 5181-1273)
CFT device:	2-way unpurged splitter capillary flow technology (Agilent p/n G3181B)
Carrier:	Hydrogen 85 cm/s, constant flow 3.5 mL/min
Injection:	1.0 µL splitless; 250 °C, Purge flow 50 mL/min at 0.3 min, Gas saver 50 mL/min at 2 min
Column 1:	Agilent J&W DB-35 ms 30 m × 0.25 mm, 0.25 µm (Agilent p/n 122-3832)
Column 2:	Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm (Agilent p/n 122-1236)
Oven:	110 °C (0.1 min), 25 °C/min to 200 °C (0.5 min), 10 °C/min to 240 °C (0.5 min), 30 °C/min to 325 °C (1.5 min)
Injection:	1µL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual µECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Table 2. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial caps:	Blue screw caps (Agilent p/n 5182-0717)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
CFT fittings:	Internal nut (Agilent p/n G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier :	20x Magnifier loop (Agilent p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA), acetic acid (HAc) was from Sigma-Aldrich (St. Louis, MO, USA), and acetone was from VWR International (West Chester, PA, USA). The PCB congeners standard (RPCM-8082) and surrogate standard (ISM-320) were purchased from Ultra Scientific (N. Kingstown, RI, USA).

Solutions and Standards

A 1% acetic acid solution in ACN was prepared by adding 10 mL of acetic acid to 1 L of ACN.

The PCB stock standard solution (100 µg/mL of 19 congeners) was diluted in acetone to yield spiking solutions of 1 and 5 µg/mL. A 10 µg/mL surrogate spiking solution was prepared by diluting the stock surrogate (200 µg/mL) solution in acetone. The spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution.

Sample Preparation

A Swai fish sample was purchased from a local grocery store. The fish was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method followed by dSPE [5]. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

A 3.0 g sample of fish was weighed into a centrifuge tube. QC samples were fortified with appropriate amount of PCB spiking solution to yield QC samples with concentrations of 10, 50, and 200 ng/mL. A 150-µL amount of surrogate spiking standard (10 µg/mL) was added to each QC sample to yield a 100 ng/mL concentration. Each sample received a 12.0-mL aliquot of deionized water and 15-mL aliquot of 1% HAc in ACN. The samples were vortexed at 1500 rpm for 1 minute. Two ceramic bars (Agilent p/n 5982-9313) were added to each sample to aid in sample extraction. An Agilent Bond Elut QuEChERS AOAC extraction salt packet (Agilent p/n 5982-5755) containing 6 g of $MgSO_4$ and 1.5 g sodium acetate was added to each centrifuge tube. The capped tubes were shaken on a Geno/Grinder at 1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 minutes.

An 8-mL aliquot of the upper layer was transferred to an Agilent Bond Elut QuEChERS fatty sample dispersive SPE 15 mL tube (Agilent p/n 5982-5158). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 5 minutes to complete the sample extraction. The liquid from the dSPE tube was transferred to a GC vial and analyzed by GC-µECD using the chromatographic conditions listed in Table 1.

Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as reagent blanks.

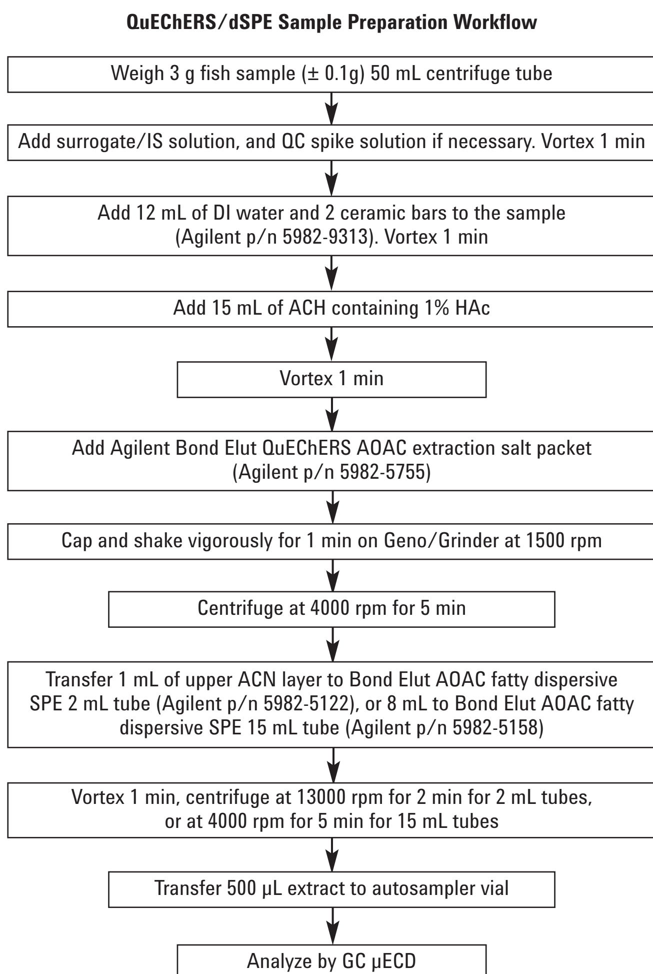

Figure 1. Flow chart of the Agilent Bond Elut QuEChERS modified AOAC extraction procedure for fish sample [5].

Table 3. r^2 Values for the PCB Congeners Calibration Standards over the 10 ng/mL to 400 ng/mL Range of this Study

Analytes	DB-35ms r^2	DB-XLB r^2
IUPAC 1	0.9994	0.9994
Tetrachloro-m-xylene (surrogate)	0.9913	0.9923
IUPAC 5	0.9993	0.9998
IUPAC 18	0.9998	0.9995
IUPAC 31	0.9980	0.9984
IUPAC 52	0.9986	0.9992
IUPAC 44	0.9988	0.9993
IUPAC 66	0.9990	0.9993
IUPAC 101	0.9992	0.9994
IUPAC 87	0.9984	0.9991
IUPAC 110	0.9939	0.9991
IUPAC 151	0.9998	0.9996
IUPAC 153	0.9981	0.9993
IUPAC 141	0.9993	0.9998
IUPAC 138	0.9984	0.9994
IUPAC 187	0.9989	0.9996
IUPAC 183	0.9993	0.9998
IUPAC 180	0.9994	0.9998
IUPAC 170	0.9993	0.9997
IUPAC206	0.9995	0.9996
Decachlorobiphenyl (surrogate)	0.9910	0.9895

Results and Discussion

The PCB and surrogate standards were resolved on the DB-35ms 30 m × 0.25 mm, 0.25 µm primary analysis column in less than 12 min. Figure 2 shows the separation of a 50 ng/mL PCB standard solution (100 ng/mL surrogate standard). Figure 3 shows a chromatogram of the same 50 ng/mL PCB standard (100 ng/mL surrogate standard) injection on the DB-XLB 30 m x 0.25 mm, 0.50 µm confirmatory analysis column.

The performance of the dual column set yielded acceptable linearity and recovery over the calibration range of this study. The method limit of quantitation (MLQ) of 10 ppb is substantially lower than the current regulatory guideline set by the FDA of 2,000 ppb for PCBs in food grade fish, and below the Agency for Toxic Substance and Disease Registry (ATSDR) maximum residue limit (MRL) of 0.02 mg/kg/day for these analytes [6]. The linearity of the column set as defined by the r^2 values of the PCB congeners standard curve ranged from 0.9939-0.9998. The individual PCB congener values are shown in Table 3. The lowest calibration standard on the column set also achieved excellent signal-to-noise ratios as shown in Figure 4.

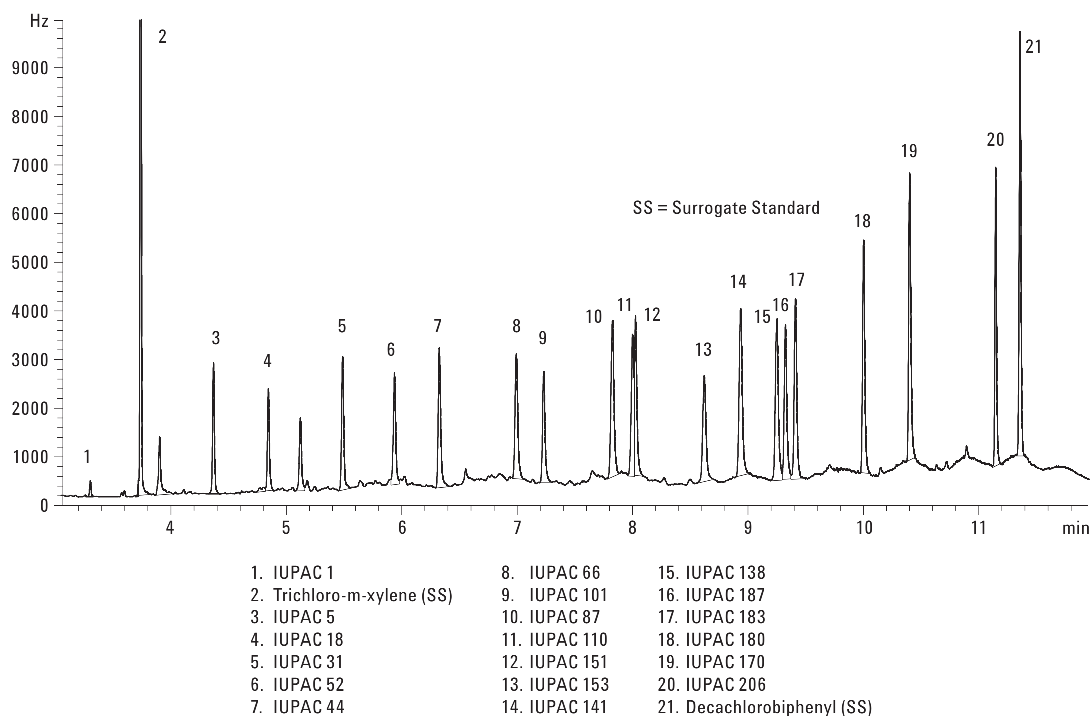

Figure 2. GC/µECD chromatogram of the 50 ng/mL PCB congeners standard analyzed on an Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 µm capillary GC column (Agilent p/n 122-3832). Chromatographic conditions are listed in Table 1.

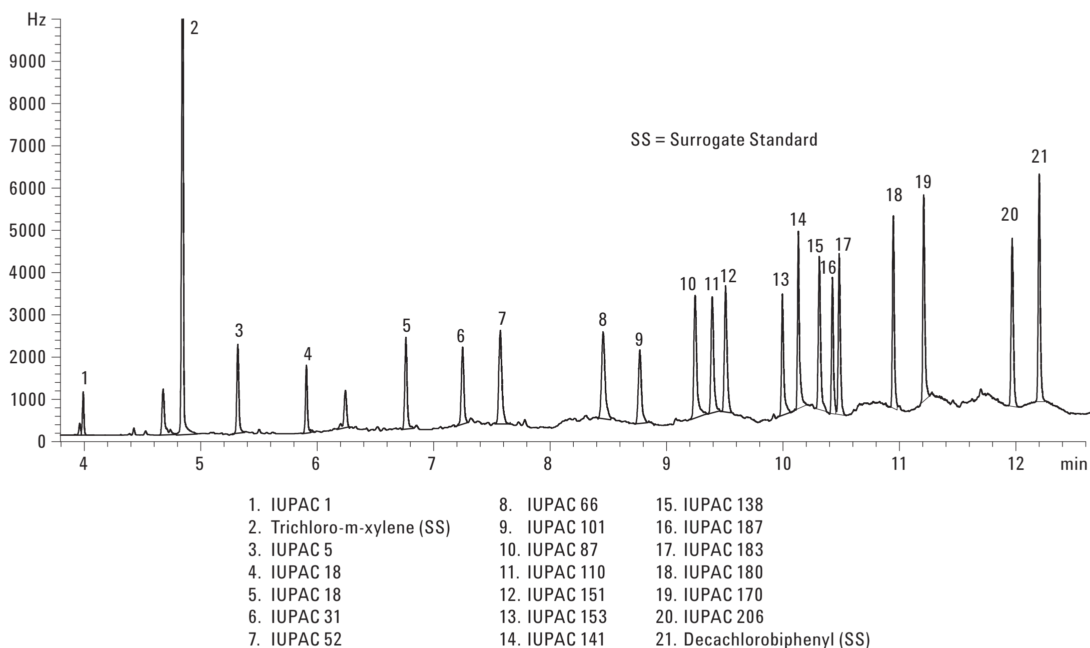

Figure 3. GC/μECD chromatogram of the 50 ng/mL PCB congeners standard analyzed on an Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 μm capillary GC column (Agilent p/n122-1236). Chromatographic conditions are listed in Table 1.

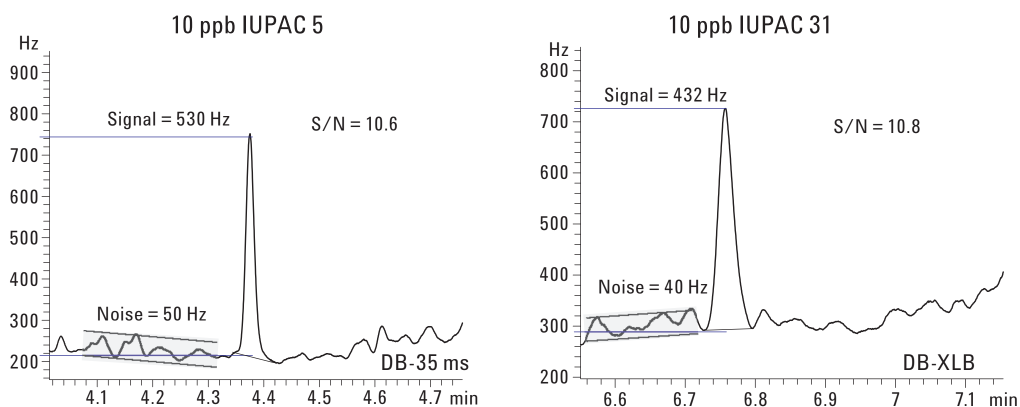

Figure 4 Enlarged view chromatogram of two individual congeners in the 10 ng/mL PCB calibration standard analyzed on the Agilent J&W DB-35ms and DB-XLB capillary columns. Chromatographic conditions are listed in Table 1.

The extraction process using the QuEChERS followed by dispersive SPE was effective in retaining the PCBs in the spiked fish sample and providing sufficient cleanup of the sample matrix for GC-µECD analysis. Figure 5 shows the separation of the extracted PCBs in a spiked fish sample on the primary and confirmation column set.

50 ppb PCBs in fish after Agilent's Bond Elut QuEChERS extraction and dispersive SPE for fatty samples

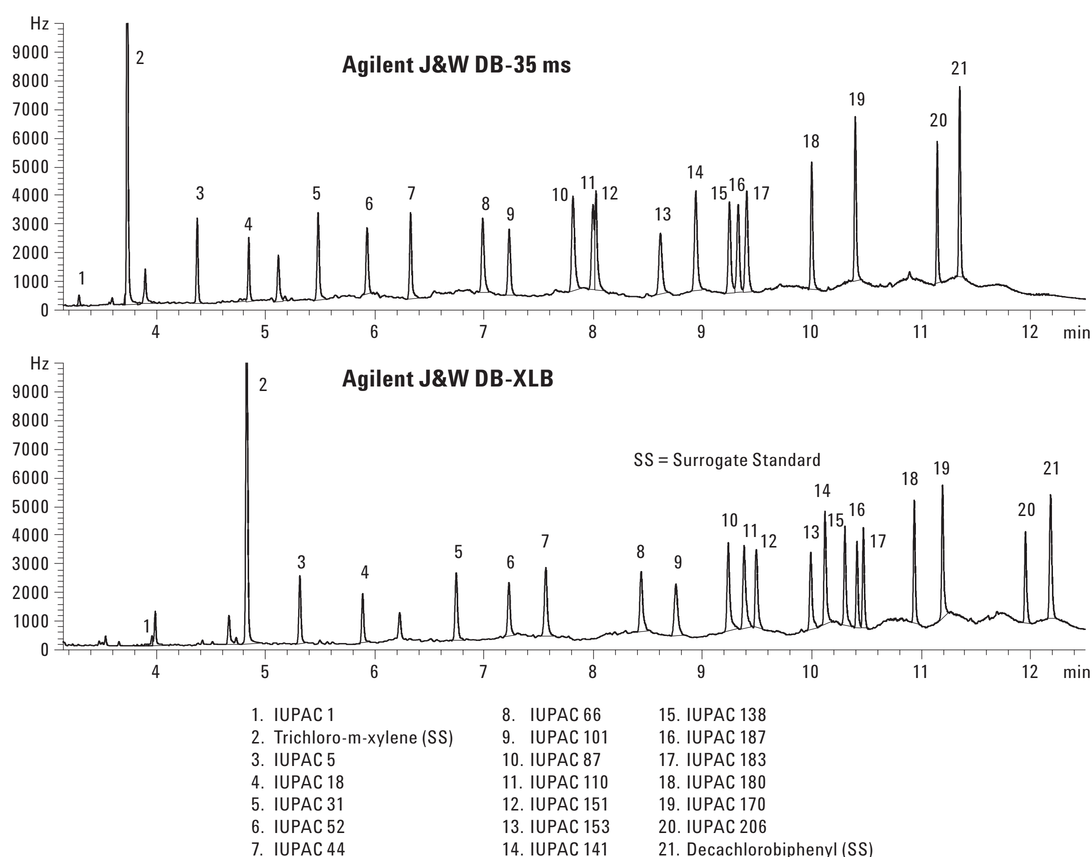

Figure 5. GC/µECD chromatogram of the 50 ng/mL fortified fish extract analyzed on Agilent's J&W DB-35ms and DB-XLB GC columns. Chromatographic conditions are listed in Table 1.

The recoveries were determined at the 10, 50, and 200 ng/mL PCB levels. Recoveries for the individual PCBs on each column are shown in Tables 4 and 5. The mid and high level recovery ranges were excellent with the DB-35ms column (72 to 112%), and with the DB-XLB column (72 to 116%) for all PCBs investigated.

Lower recoveries were noted for three of the 19 congeners in the low level 10 ppb QC sample. The co-elution of IUPAC 110 and IUPAC 151 on the DB-35ms column contributed to the lower recovery seen for the IUPAC 110, but both congeners were resolved on the XLB column yielding recoveries over 72%.

Because the sensitivity of the ECD is relative to the amount of chloro substituents present, the response for the PCBs generally increases with increased chlorine content. Since IUPAC 1 is a monochlorinated biphenyl (2-chlorobiphenyl) it exhibits poor ECD sensitivity. This was found to contribute to the low recovery for IUPAC 1 at the 10 ng/mL level, however recovery and reproducibility were excellent at 50 ng/mL (average recovery 111%, average reproducibility 2.4%).

Table 4. Recovery and Repeatability of PCBs in Fortified Swai Fish with Agilent J&W DB-35ms column (Agilent p/n122-3832)

	10 ng/mL fortified QC		50 ng/mL fortified QC		200 ng/mL fortified QC	
Analytes	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**
IUPAC 1	43.2	4.0	111.9	2.4	99.0	1.6
Tetrachloro-m-xylene (surrogate)	107.5	1.2	110.4	1.4	103.9	1.8
IUPAC 5	68.5	1.3	107.0	1.8	99.6	2.2
IUPAC 18	99.3	2.9	108.5	1.9	95.1	2.5
IUPAC 31	56.7	3.3	110.5	2.0	101.8	2.3
IUPAC 52	75.7	3.4	91.8	2.1	100.5	1.9
IUPAC 44	61.4	1.7	107.8	2.2	100.6	2.1
IUPAC 66	66.9	2.0	97.2	2.8	96.2	2.4
IUPAC 101	65.8	2.5	99.5	2.8	94.7	2.2
IUPAC 87	75.7	2.8	99.1	1.5	101.4	2.4
IUPAC 110	29.7	2.6	100.0	2.6	102.8	2.8
IUPAC 151	100.1	2.1	99.8	2.6	94.5	1.9
IUPAC 153	49.9	1.9	89.9	1.6	91.5	2.9
IUPAC 141	67.7	2.1	93.1	1.5	92.2	2.3
IUPAC 138	52.2	2.9	95.9	2.4	93.4	2.2
IUPAC 187	57.0	3.4	92.1	2.3	89.5	2.2
IUPAC 183	62.1	3.2	87.3	2.2	85.7	2.2
IUPAC 180	63.1	3.2	88.6	1.1	84.0	2.6
IUPAC 170	65.4	4.2	91.0	1.6	86.7	2.3
IUPAC 206	58.8	3.2	77.7	1.5	72.5	2.4
Decachlorobiphenyl (surrogate)	75.0	2.9	81.1	2.4	75.9	2.7

Table 5. Recovery and Repeatability of PCBs in Fortified Swai Fish with Agilent J&W DB-XLB column (Agilent p/n 122-1236)

	10 ng/mL fortified QC		**50 ng/mL fortified QC**		**200 ng/mL fortified QC**	
Analytes	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**
IUPAC 1	56.5	3.2	116.2	2.2	99.4	2.0
Tetrachloro-m-xylene (surrogate)	108.0	1.6	110.9	1.3	105.1	1.9
IUPAC 5	87.4	2.5	112.1	1.5	100.7	1.9
IUPAC 18	69.9	1.6	112.3	2.1	100.8	2.5
IUPAC 31	63.1	3.5	108.3	1.8	103.2	2.3
IUPAC 52	59.6	3.5	104.7	2.6	100.5	1.8
IUPAC 44	75.7	2.9	105.0	1.9	101.7	2.0
IUPAC 66	83.1	2.9	101.5	2.7	98.2	2.0
IUPAC 101	73.0	2.4	98.4	1.9	96.6	2.0
IUPAC 87	60.7	2.7	109.2	1.8	100.7	1.6
IUPAC 110	72.9	2.6	103.0	2.3	100.1	1.6
IUPAC 151	75.1	3.6	93.9	2.9	93.5	2.6
IUPAC 153	36.0	6.9	104.5	3.0	91.1	1.9
IUPAC 141	74.4	2.6	98.0	1.6	91.9	2.3
IUPAC 138	65.4	3.3	98.9	2.5	93.1	2.1
IUPAC 187	68.4	2.2	94.6	1.0	88.7	2.0
IUPAC 183	72.6	3.7	92.2	2.0	86.1	2.4
IUPAC 180	76.0	3.2	92.2	2.0	84.5	2.7
IUPAC 170	74.9	8.7	94.6	2.2	87.5	2.3
IUPAC 206	60.1	3.2	78.3	2.4	72.6	2.7
Decachlorobiphenyl (surrogate)	74.6	2.4	81.4	1.6	76.7	2.3

Conclusions

This application note shows a robust, inexpensive, analytical method that sufficiently monitors PCBs in fish samples to address food safety concerns. This method demonstrates the feasibility of using a dual column µECD approach for routine fish screening as an alternative to GC/MS.

The Agilent Bond Elut QuEChERS AOAC method for fatty samples followed by dSPE is effective at providing enough sample cleanup to avoid matrix interferences, while maintaining low level analyte detection.

The dual column set of an Agilent J&W DB-35ms primary analytical column and an Agilent J&W DB-XLB confirmatory column on one instrument allows simultaneous identification and confirmation of the presence of the PCBs. The DB-35ms primary analysis column and the DB-XLB confirmatory column with dual µECD detection were effective at analyzing 19 PCBs in a fish matrix following sample matrix cleanup. The single injection, dual column approach improves productivity by saving instrument and analyst time. Continuous improvements and stringent process control with respect to column activity make the DB-35ms and DB-XLB column pair an excellent choice for analysis of analytes such as PCBs.

The performance of the dual column set DB-35ms and DB-XLB with GC µECD had excellent linearity over the range of concentrations studied with r^2 values between 0.9939 and 0.9998 for the PCB compounds. Recovery and reproducibility was shown to be greater than 77% with an RSD below 3.0 at 50 ng/mL. The method limit of quantitation for the PCB congeners using this approach was significantly lower than currently regulated MRLs in fish. The results achieved with this method shows determination of PCBs by µECD as a reliable alternative to GC/MS.

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