Direct Injection of Fish Oil for the GC-ECD Analysis of PCBs: Results Using a Deans Switch With Backflushing

Application

Environmental and Pharmaceutical

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Abstract

A Deans switch, employing Agilent's Capillary Flow Technology, was configured on an Agilent 7890A gas chromatograph (GC) equipped with dual electron capture detectors (ECDs). A method was developed for the analysis of fish oil for polychlorinated biphenyl (PCB) contamination. The Deans switch was used to heart cut 7 indicator PCBs (IUPAC congeners 28, 52, 101, 118, 138, 153, and 180) from the primary DB-XLB column onto a DB-200 column for further separation. Fish oil from a supplement capsule was simply diluted 1:10 in isooctane and injected directly. In a separate experiment, the fish oil was analyzed by GC with a flame ionization detector (GC/FID) without backflushing. From these analyses, it was estimated that about two-thirds of the fish oil components would remain on the column after the 17.4-minute GC/ECD run. To prevent carryover, contamination, and retention time shifts, the Deans switch was used to backflush the primary column at the end of each run. Evidence shows that backflushing removed the fish oil residue, which otherwise would quickly degrade the chromatography.

Introduction

Fish oils contain high levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), omega-3 fatty acids that are thought to have beneficial health affects. In addition to eating fish, many people take fish oil as a supplement to their daily diet. However, fish, especially those high on the aquatic food chain, can bioaccumulate fat-soluble pollutants. Among these are polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Therefore, fish oil used in supplements undergoes a variety of analyses, including tests for halogenated pollutants.

One of the quality assurance tests is to analyze fish oil for PCB contamination. This is complicated by the fact that fish oil is a very complex mixture containing high-boiling fatty acids and triglycerides of fatty acids; chain lengths are mostly between 14 and 22 carbons. They also contain varying amounts of phospholipids, glycerol ethers, wax esters, and fatty alcohols. PCB analysis is complex by itself, with 209 possible congeners. Of these, 140 to 150 have been observed in commercial PCB mixtures called Aroclors. PCB analysis usually focuses on the 12 planar, dioxin-like PCBs and/or on seven indicator PCBs (IUPAC Numbers 28, 52, 101, 118, 138, 153, and 180).

To obtain sufficient sensitivity and selectivity for these compounds, analysts have traditionally employed very expensive techniques such as high-resolution mass spectrometry (HR/MS) or HR/MS/MS. Analysis of the fish oil generally follows a series of extraction and cleanup steps. This paper focuses on the analysis of the seven indicator PCBs in fish oil using an Agilent 7890A GC configured with a Deans switch, two columns of differing selectivity, and dual electron capture

detectors (ECDs). Fish oil from a commercially available supplement was simply diluted 10:1 in isooctane and injected into the GC. No cleanup steps were employed.

Experimental

The fish oil supplement was obtained from a local grocery store. According to the bottle's label, each gelatin capsule contains 1.0 g of fish oil of which 180 mg is EPA and 120 mg is DHA. Oil was removed from a capsule and diluted with isooctane (pesticide grade from Sigma-Aldrich, Milwaukee, WI, USA) to make a 10% solution. This solution was spiked with various Aroclors (Supelco, Bellefonte, PA, USA) or with individual PCB congeners (AccuStandard, New Haven, CT, USA).

Table 1 lists the instrumentation and experimental conditions for the analysis.

Table 1. Instrumentation and Experimental Conditions

Instrumentation and Software	
Gas chromatograph	Agilent 7890A
Automatic sampler	Agilent 7683B Series injector and tray
Primary column	J&W 30-m × 0.18-mm × 0.18-µm DB-XLB (P/N 121-1232)
Primary column connections	Split/splitless inlet to Deans switch
Secondary column	J&W 30-m × 0.25-mm × 0.50-µm DB-200 (P/N 122-2033)
Secondary column connections	Deans switch to back ECD
Restrictor	76.8-cm × 0.100-mm deactivated fused silica tubing
Restrictor connections	Deans switch to front ECD
Inlet liner	Agilent deactivated single taper with glass wool (P/N 5062-3587)
Auxiliary pressure control device	Agilent 7890A Pneumatic Control Module (PCM) Option # 309
Deans switch calculator software	Agilent Technologies Deans Switch Calculator (Rev. A.01.01)
Software for data acquisition and analysis	Agilent GC ChemStation (Rev. B.03.01)
Instrumental Conditions for Analysis	
Inlet	Split/splitless at 330 °C
Oven temperature program	80 °C (1 min), 50 °C/min to 200 °C (0 min), 10 °C/min to 290 °C (5 min)
Detectors	Dual ECD at 340 °C
ECD make-up gas	N_2 at 60 mL/min
Inlet pressure	H_2 at 41.040 psig (constant pressure mode)
PCM pressure to Deans switch	H_2 at 20.610 psig (constant pressure mode)
Post-Run Backflush Conditions	
Post-run duration	2.4 min
Inlet pressure	H_2 at 0 psig
PCM pressure	H_2 at 80 psig
Oven temperature during backflush	290 °C for 2.4 min

Results and Discussion

Without backflushing, the high-boiling components of fish oil can be retained by the GC column, causing severe carryover problems from one run to the next. After a few injections, so much of the fish oil residue builds up on the column that it causes PCB retention times to shift by a minute or more. Such dramatic retention time shifts would prevent the use of the Deans switch, where heart cuts are just a few seconds wide.

Deans Switch–Heart Cutting

The Deans switch is one of Agilent's new devices that employ Capillary Flow Technology. These devices have extremely low dead volumes, are inert, and do not leak, even with large cycles in oven temperature. Columns are easy to install into the Deans switch, which is mounted on the side of the oven wall (Figure 1).

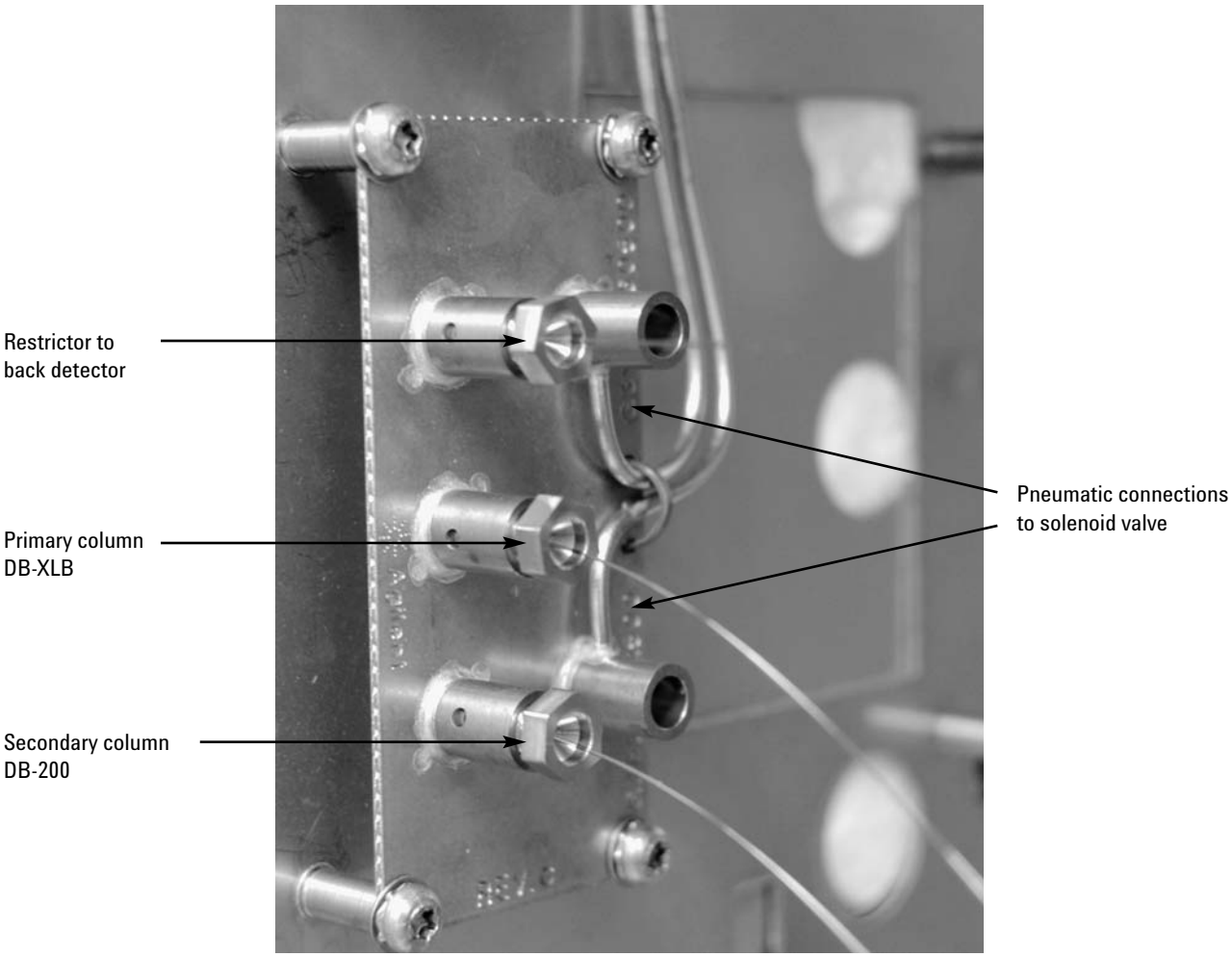

Figure 1. Photograph of the Deans switch installed on the side of the 7890A GC oven. The column and restrictor connections are indicated by an * in Figure 2a.

As shown in Figure 2a, the 30-m × 0.18-mm × 0.18-µm DB-XLB column is connected between the split/splitless inlet and the Deans switch. A short length of deactivated fused silica tubing (76.8 cm × 0.100 mm) connects the Deans switch to the front ECD. The secondary column (30-m × 0.25-mm × 0.5-µm DB-200) was chosen because it is more polar than the DB-XLB column and has a different selectivity for PCBs. It has an upper temperature limit of 300 °C, which is high enough to elute the PCBs of interest.

Figure 2a shows the Deans switch in the "normal" mode with the solenoid valve in the off position. In this mode, the effluent from the primary DB-XLB column is directed through the restrictor to the front ECD. When the solenoid valve is switched, the effluent is directed through the secondary DB-200 column to the back ECD (Figure 2b). The retention times for the seven indicator PCBs were initially determined with the valve in the *off* position. Using the timed events table in the ChemStation, the valve was switched to *on* just before each PCB peak and *off* immediately after. This produced seven heart cuts that were directed through the DB-200 column to the back ECD.

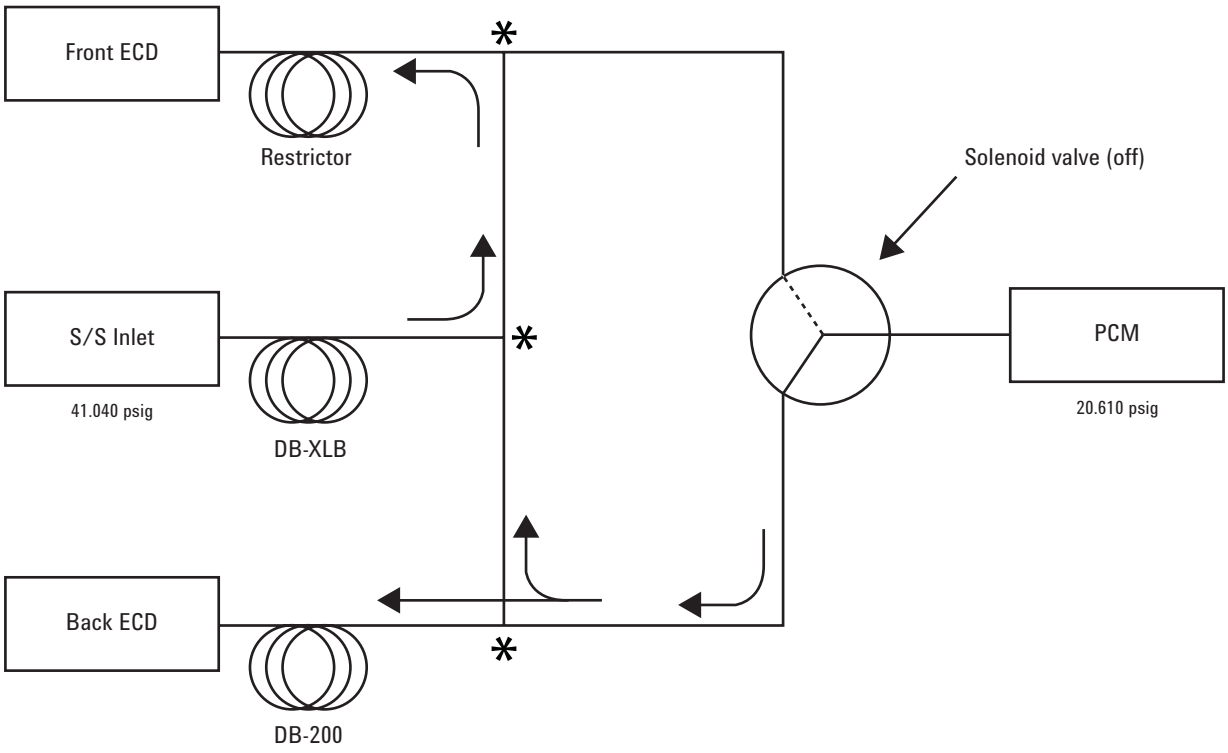

Figure 2a. Deans switch in the "no cut" position. The effluent from the DB-XLB column goes directly to the front ECD through the short restrictor. The intersections marked with an * are column and restrictor connections to the Deans switch plate (Figure 1).

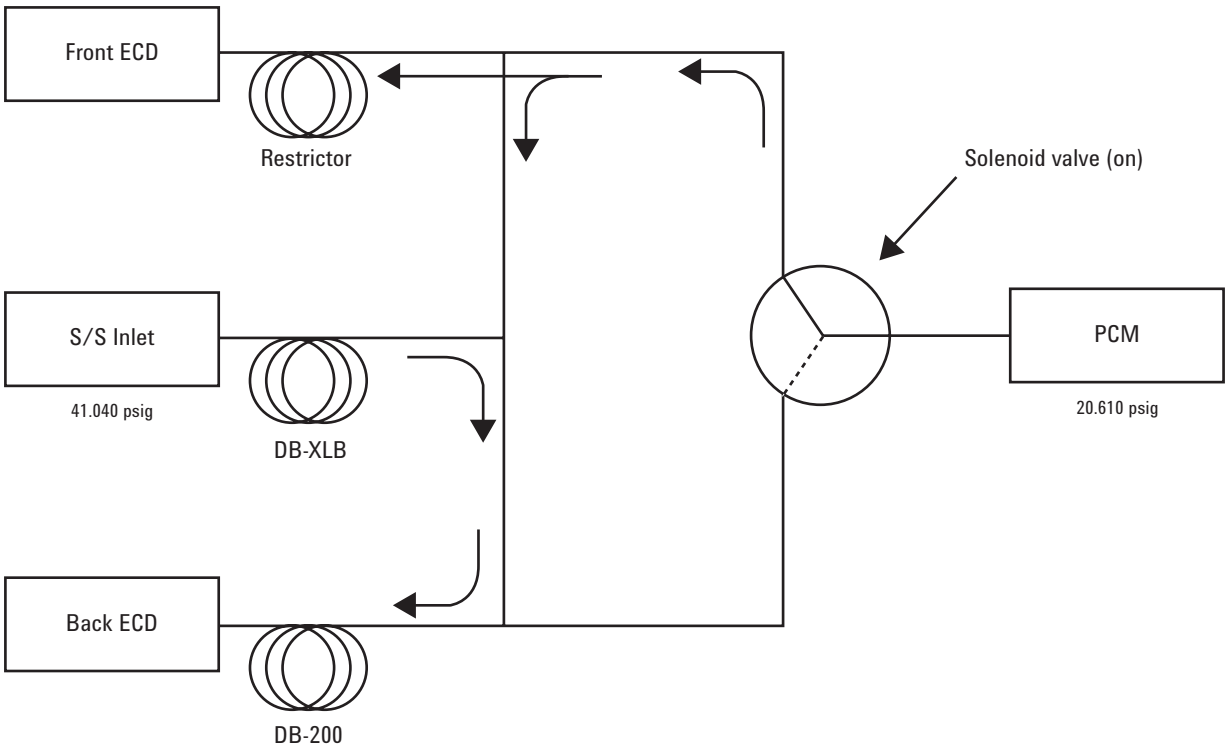

Figure 2b. Deans switch in the "cut" position. The effluent from the DB-XLB column goes to the DB-200 column and then to the back ECD.

In some Deans switch systems, the second column is placed in a separate GC oven or cryogenic cooling is used to trap the heart cut components at the head of the second column. In this case, both columns were mounted inside of the 7890A oven and cooling was not used to focus compounds at the head of the DB-200 column.

Figure 3a shows the chromatogram for a fish oil sample spiked with Aroclor 1260. PCBs 28, 52, 101, 118, 138, 153, and 180 were cut out of the primary chromatogram (Figure 3b) and sent to the second column (Figure 3c). The purpose of the DB-200 column is to resolve the target PCBs from other PCBs and matrix components that co-elute with them on the DB-XLB column. Six of the 7 PCBs appear to be well resolved on the DB-200 column. PCB 118 is only partially resolved by this method.

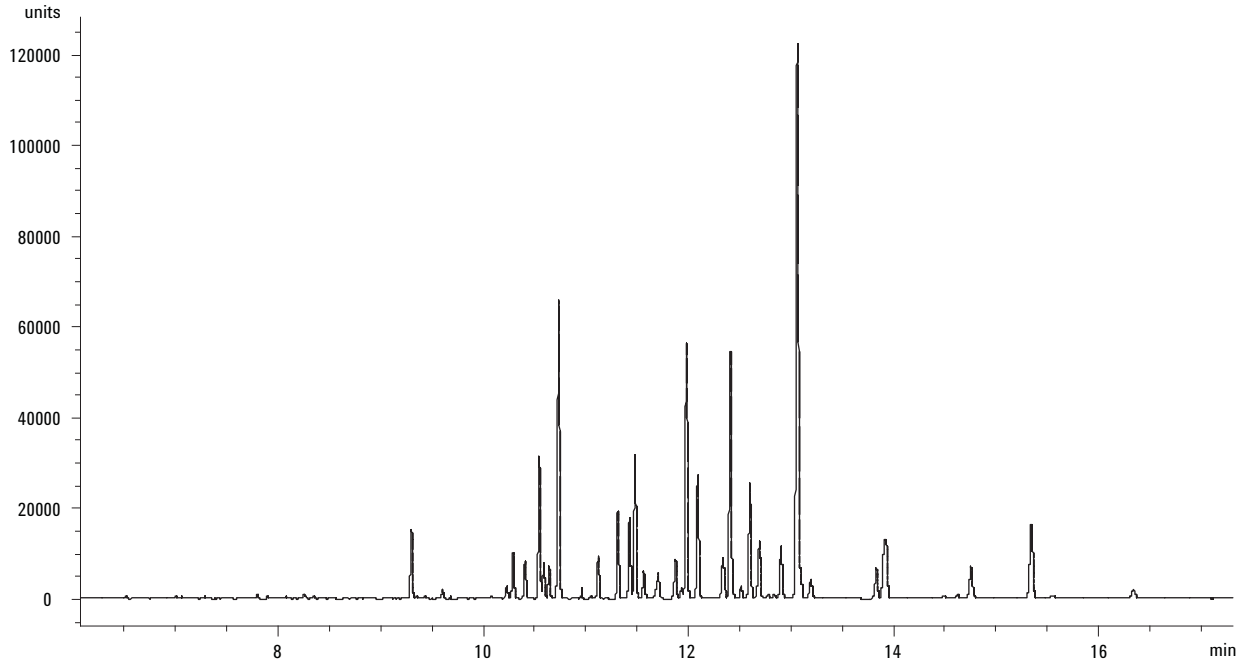

Figure 3a. GC/ECD chromatogram of Aroclor 1260 spiked into fish oil. This is the effluent from the primary DB-XLB column with seven heart cuts.

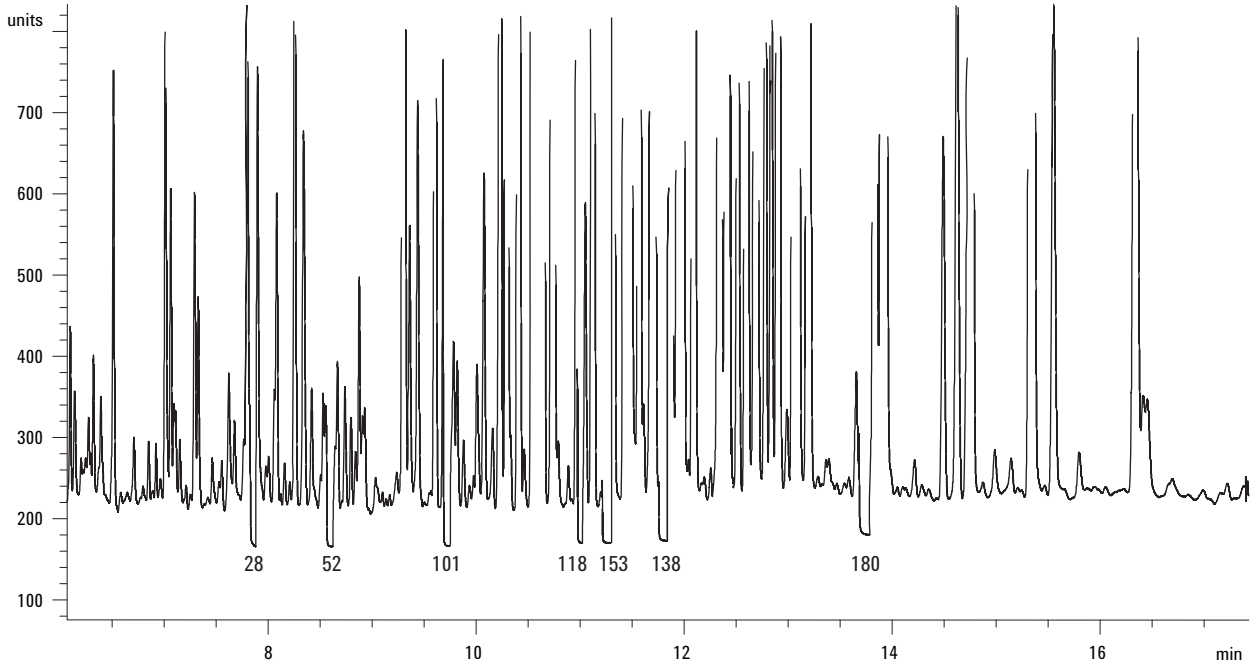

Figure 3b. Enlargement of the chromatogram in Figure 3a showing where heart cuts were made for the seven target PCBs.

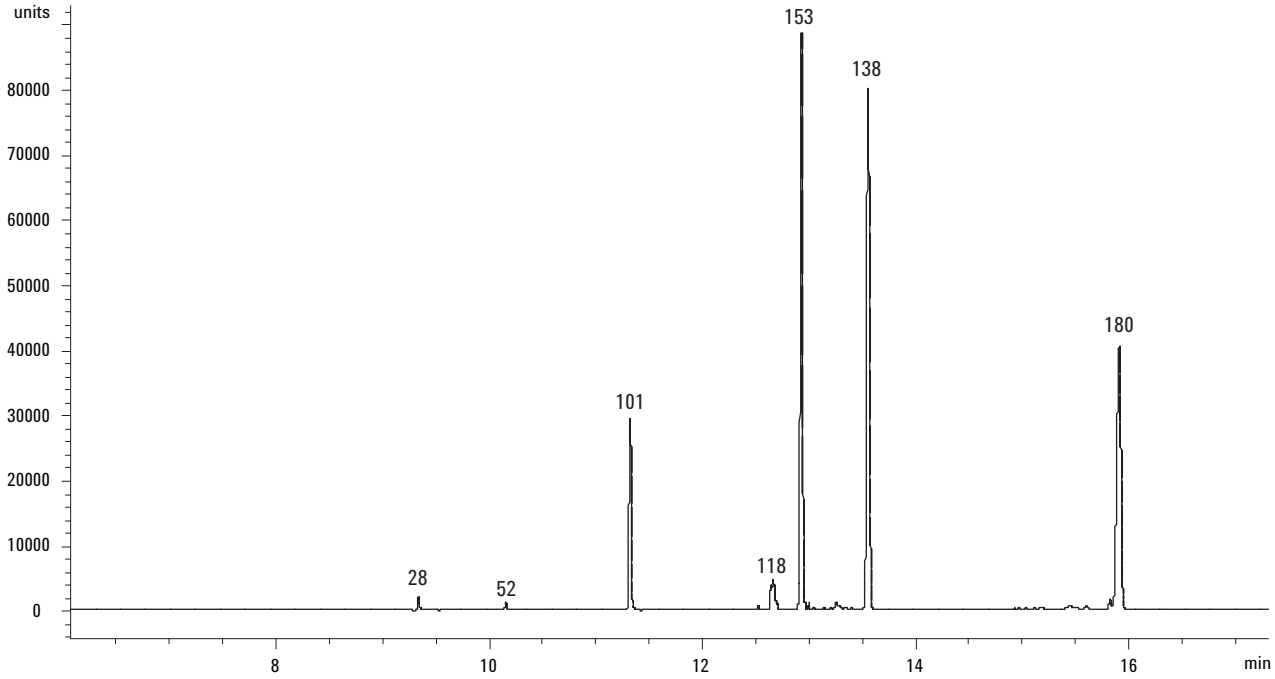

Figure 3c. GC/ECD chromatogram from the DB-200 column. The peaks in this chromatogram were heart cut from the DB-XLB column. Except for congener 118, the target PCBs were separated from co-eluting interferences by the DB-200 column.

Deans Switch–Backflushing

Data collection with the Deans switch system ended at 17.4 min with the oven at 290 °C. While it was assumed that a lot of the fish oil components remained on the column at this point, it was impossible to tell because the ECD responds poorly to these compounds. The fish oil does contribute some small peaks (both positive and negative) to the chromatogram, but it is impossible to see the full contribution of the fish oil. So a sample of the fish oil was analyzed on an identical DB-XLB column using a flame ionization detector (FID) with no Deans switch installed. The temperature was held at 290 °C for an extra 25 minutes to determine if high boiling compounds were still eluting.

Figure 4 shows that a great deal of the fish oil continued to elute after 17.4 minutes (arrow in figure). When a blank run was made with a final oven temperature of 310 °C, much more of the fish oil eluted from the column (Figure 4, middle chromatogram). A second blank run (Figure 4, top chromatogram) showed that fish oil components were still eluting from the column. In actuality, only about a third of the fish oil comes off the column under the Deans switch conditions. This is why other fish oil methods begin with a solvent extraction followed by solid phase extraction for sample cleanup.

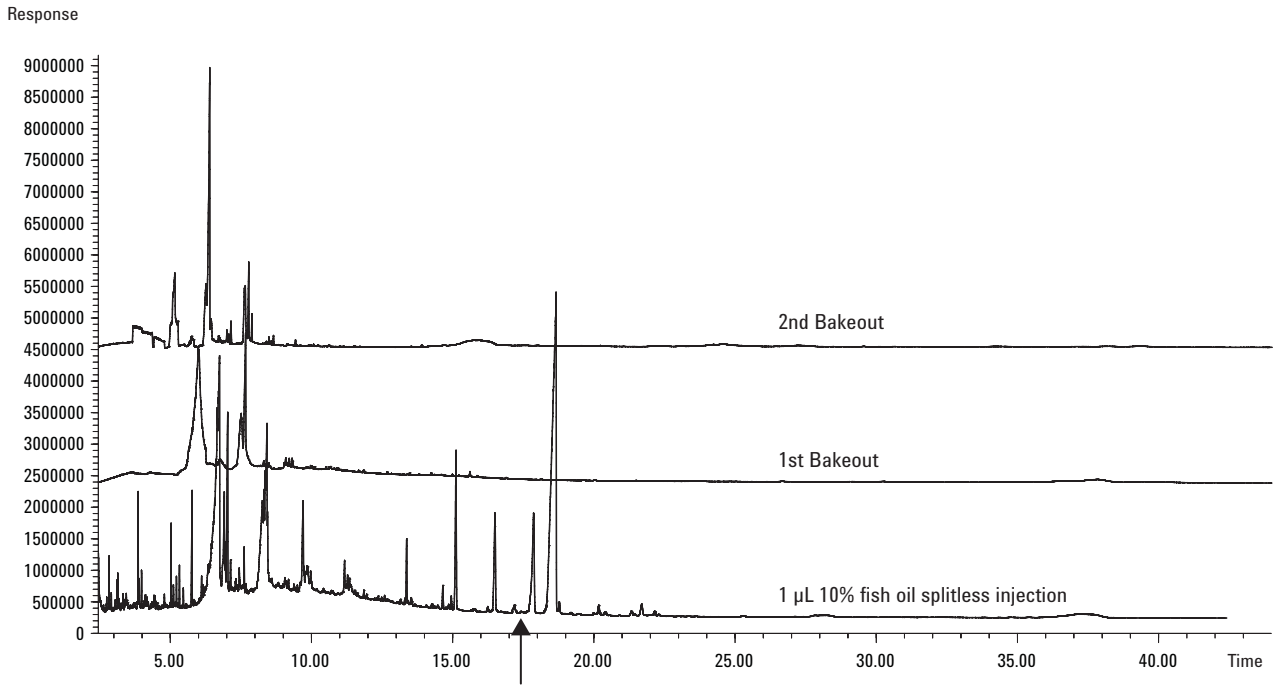

Figure 4. GC/FID chromatogram from a 1 µL splitless injection of 10% fish oil using a 30-m × 0.18-mm × 0.18 µm DB-XLB column. The arrow indicates where the GC/ECD method ends and the post-run backflush begins. In this case, there was no backflushing so the oven was held at 290 °C for an extra 25 min. The run was repeated two more times without injection but with the oven held at 310 °C for 30 minutes at the end of the run. Residue from the fish oil injection continued to elute, even during a second bakeout step.

The 7890A has been designed to make column backflushing a routine process. It has been shown empirically that backflushing should continue for about five times the holdup time. In this case the column was held at 290 °C during the post run backflush. At the same time, the inlet pressure was dropped to 0 psig while the PCM pressure was increased to 80 psig. Using Agilent's GC Pressure/Flow Calculator software, the H_2 flow rate backwards through the column was 3.81 mL/min and the holdup time was 0.466 min. Backflushing was, therefore, continued for 2.4 minutes, which is slightly more than five times the calculated holdup value. Figure 5 shows the Deans switch in the backflush mode.

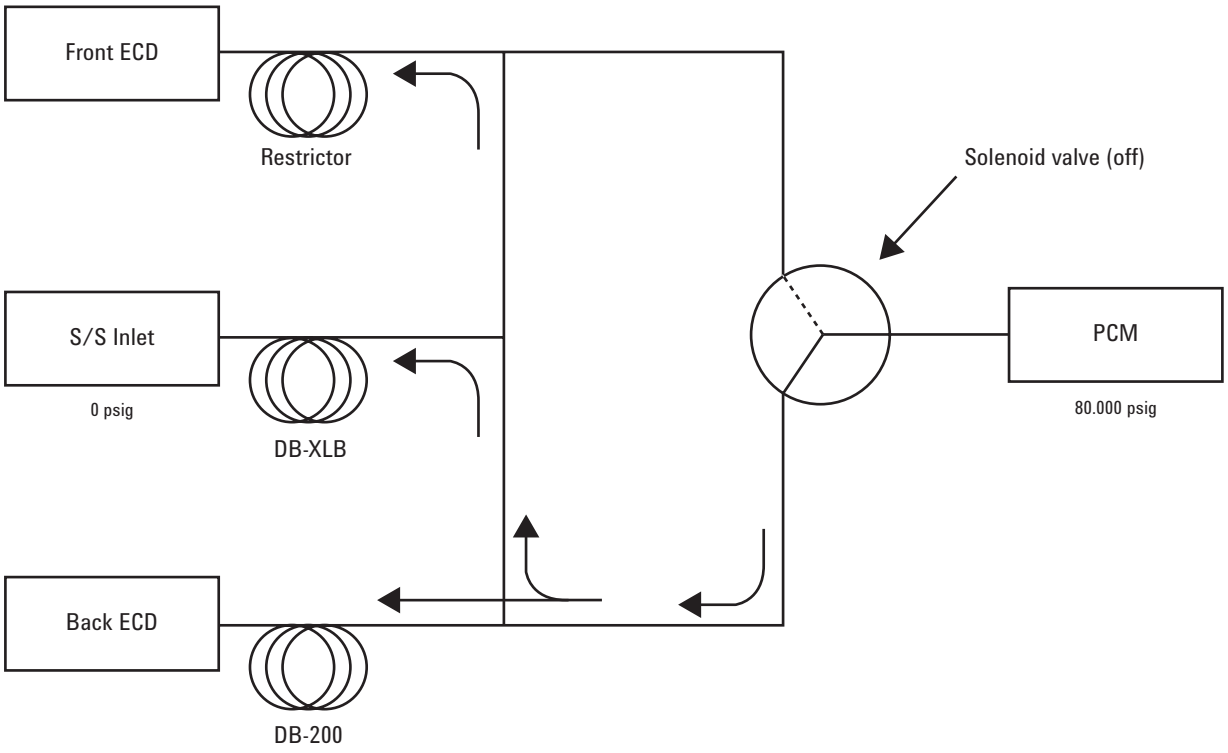

Figure 5. Deans switch in the "backflush" mode. The inlet pressure is dropped to 0 (or 1) psig while the PCM pressure is raised to 80 psig. This causes the carrier gas to flow backwards through the DB-XLB column. The reverse flow sweeps high-boiling fish oil components off the head of the column and out the split vent.

As mentioned earlier, just a few injections of fish oil can cause dramatic shifts in PCB retention times. Backflushing forces the remaining fish oil components backwards through the primary column and out through the split vent. This prevents fish oil buildup on the column, thus eliminating carryover and retention time shifts. Figure 6a compares the first and last chromatograms in a six-run sequence. One-µL splitless injections were made of 10% fish oil spiked with Aroclor 1260. This sequence was run after many previous injections of fish oil using this method, and it is clear that the retention times did not shift.

Figure 6b shows the seven PCBs that were heart cut from the two analyses shown in Figure 6a. Figure 6b shows no differences in the first and last heart cut chromatograms, providing further proof that there were not even subtle shifts in the PCB retention times. Each heart cut was just 4 to 5 seconds wide, so very small RT shifts in the first column would dramatically alter the results in the second.

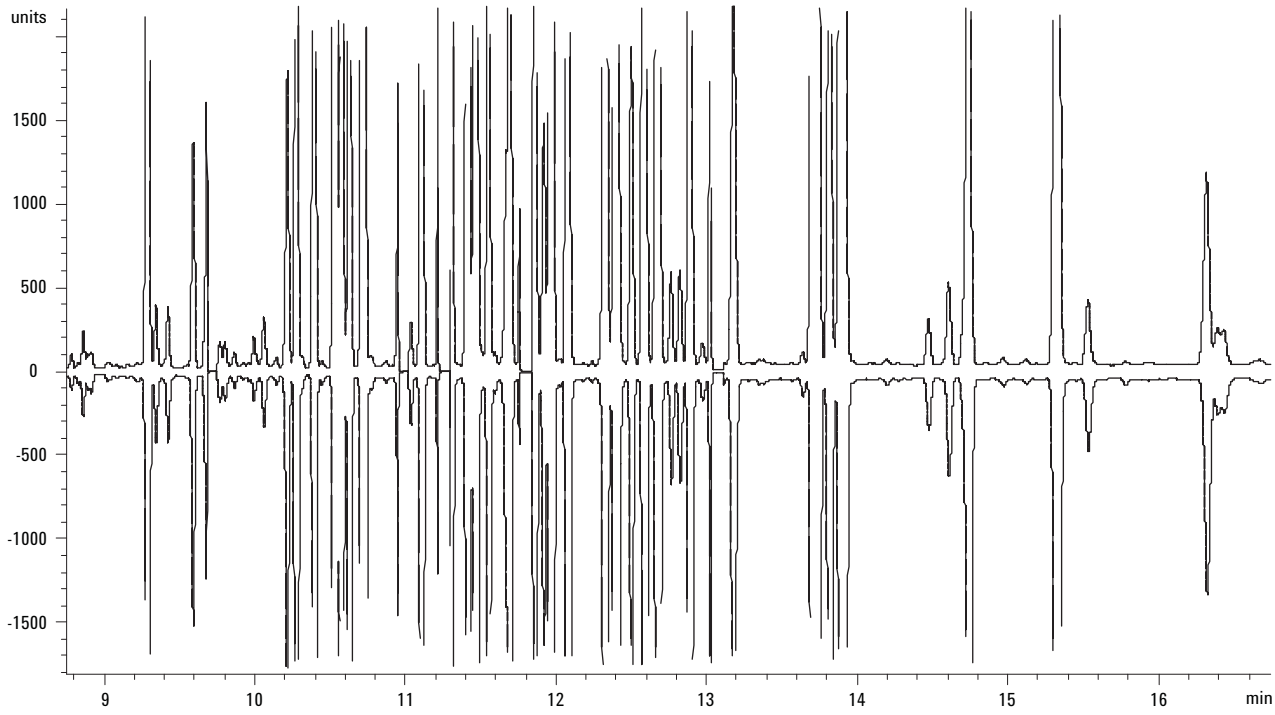

Figure 6a. First (top) and sixth (inverted) injections of 10% fish oil spiked with Aroclor 1260. Seven Deans switch cuts were made from this DB-XLB column in order to isolate PCBs 28, 52, 101, 118, 138, 153, and 180. The DB-XLB column was back-flushed after each run, preventing build-up of fish oil residue. The comparison shows that there was no shift in retention times caused by fish oil accumulation.

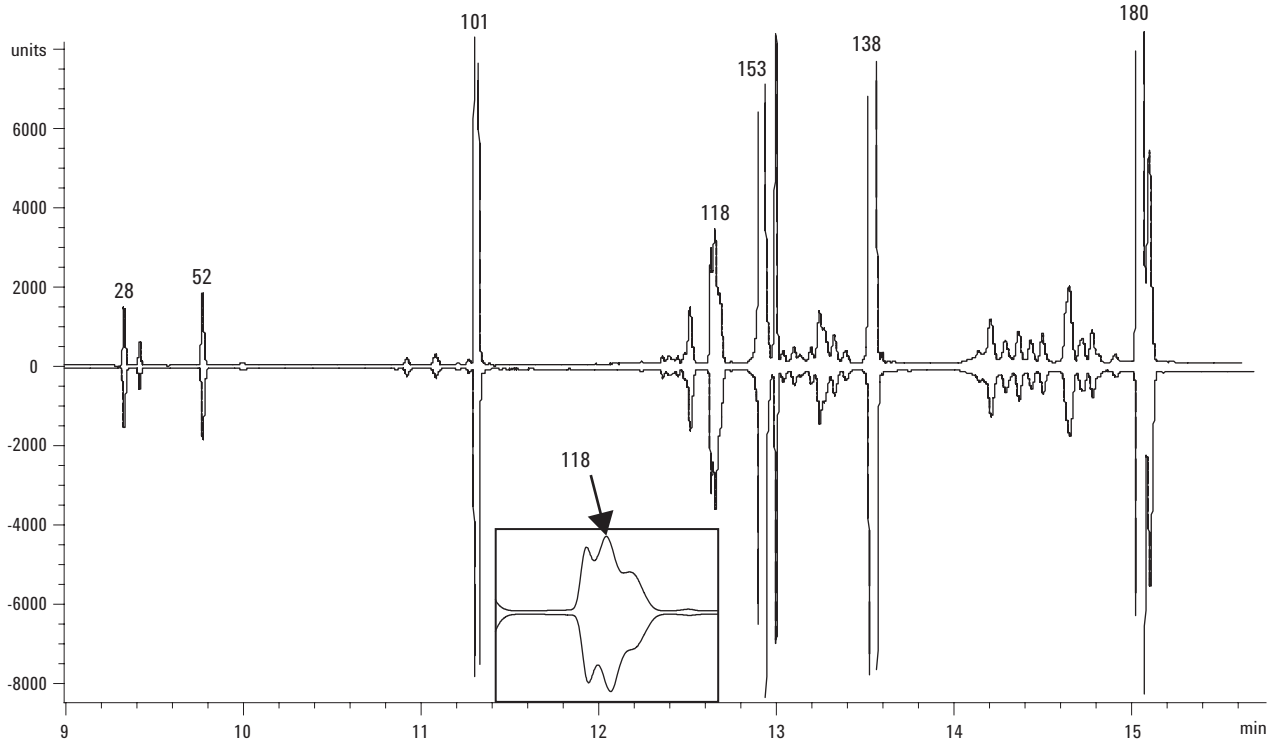

Figure 6b. Chromatogram of the seven PCB congeners and interferences that were cut from the DB-XLB column to the DB-200. The first chromatogram (top) and sixth (inverted) are identical, providing further proof of retention time stability. Any retention time shift on the primary column would severely alter the appearance of the secondary chromatogram.

Conclusions

This paper demonstrates that it is possible to analyze PCBs in fish oil without performing laborious sample cleanup prior to GC injection. A Deans switch was used to cut seven target PCBs (28, 52, 101, 118, 138, 153, and 180) from a DB-XLB column for further separation on a DB-200 column. This produced nearly baseline separation of the target PCBs. Only congener 118 was not well separated from co-eluting PCBs. Further refinement of the oven temperature program would be needed to isolate this congener.

It has been estimated that about two-thirds of the fish oil remained on the primary GC column at the end of the run. By setting the Deans switch to the backflush mode for just 2.4 minutes at the end of each run, this material was swept backwards through the column and out the split vent. There was no evidence for retention time shifts or carryover from run to run.

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