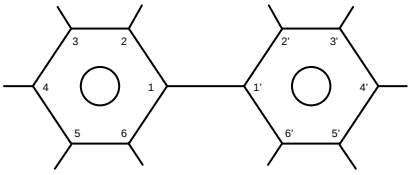
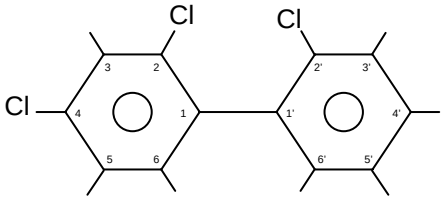
PCB Pattern Matching Program for Varian Star Workstation

Polychlorinated Biphenyls (PCBs) present an unusual analytical problem in measuring their concentrations. PCBs are two benzene rings with various numbers of chlorines substituted in any of ten positions on the two rings. The permutation of 10 chlorines yields 3,628,800 (10!) possible variations. Since most of them are mirror images of others, these duplicates are identical structurally and are not consider as different compounds. Only 209 are deemed separate chemical species and are labeled as PCB congeners. The structure of the PCB backbone is:



One example of a congener is 2, 2', 4 triChloro Biphenyl; its structure is:



Commercial PCB products[1] were comprised of a mixture of these congeners. Aroclor is the brand name for a series of products made by Monsanto. Figure 1 illustrates the many congeners detectable within a typical capillary chromatogram from an Aroclor. The analysis for PCBs becomes more difficult than the routine quantitation process. With PCBs, a single chromatographic peak does not represent the whole concentration. All congeners need be measured and included in the result to yield the total value. However, identifying and quantitating each congener is very demanding analytically, with the many possible congener standards needed for full characterization.

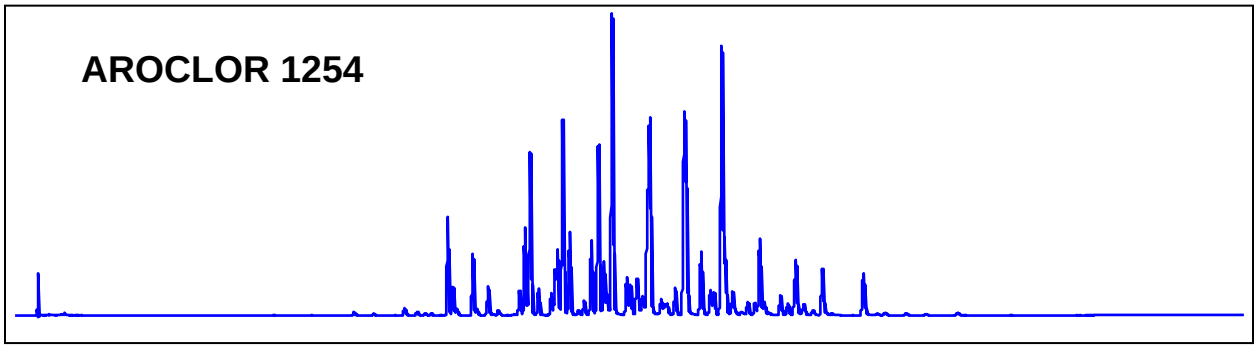


Figure 1. Typical capillary chromatogram for Aroclor 1254, showing 55 sizeable peaks.

[1] PCBs are no longer manufactured in the United States after 1977 due to their potential environmental damage caused primarily from their extreme stability in the environment.

Fortunately, the manufacturing process for generating PCBs yielded very consistent congener mixtures for each commercial product. In the United States, nearly all PCBs were sold under the brand name Aroclor, with the numerical designation usually representing the percentage (by weight) of chlorine.[2] For example, Aroclor 1260 has 60% chlorine in the mixture. With each specific Aroclor possessing very similar congener composition, its chromatographic pattern will always be characteristic of that Aroclor. By comparing the pattern of an unknown with various Aroclor standards, the Aroclor can be identified. Concentration is computed by comparing the relative peak sizes of the unknown to the standard.

Various approaches to this pattern matching have been proposed. The simplest has been a visual comparison of the chromatogram for the unknown with standards. Often a conventional glass window has been employed to visualize the overlay. Once the match is made, selected peaks are then employed in the quantitation and the results are averaged to derive the final result. This approach has worked for very experienced operators, but fails when mixtures are encountered or when pesticides are mixed in with the Aroclor. In addition, the process is not well suited to full automation and is very subjective with different operators yielding different results. Another method originally proposed by Webb and McCall[3] involves a "truth" table where the presence or absence of specific peaks leads toward a possible Aroclor. The same difficulties occur here, as well, with mixtures and pesticide interferences. Also, the table is set for very specific analytical conditions, including a particular chromatographic column. And, finally EPA Methods 8082 and 600/4-81-045 are very generic in their approach to identifying the Aroclor; they only indicate that the pattern should be matched with standards; quantitation is performed by averaging results for selected peaks.

To overcome these limitations, a novel approach to identify and quantitate Aroclor mixtures in transformer oils was developed by Lea, Bramston-Cook and Tschida.[4] Its latest version utilizes chromatographic data from the Varian Star Workstation. Retention times and raw area counts for known Aroclor standards are collected and stored for subsequent comparisons. When unknown samples are examined, their peak areas are ratioed with standard areas matched by retention times. If these ratios are consistent with a given Aroclor, then a perfect match is confirmed. Figure 2 illustrates a good consistency across a region for a comparison of Aroclor 1254 and a sample. Figure 3 compares Aroclor 1260 with a sample for the same region, showing a complete mismatch, with most sample peaks in this region missing in Aroclor 1260.

The concentration for the unknown is reported from the average of the ratios when peaks demonstrate consistency. Interfering pesticides and other non-"Arolcor" peaks are mathematically excluded by virtue of these peaks not being in the standards or of their wildly excessive ratios.

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[2] Aroclor 1016 is one mixture that does not fit into this convention.

[3] Webb, R. G., McCall, A. C., "Identities of Polychlorinated Biphenyl Isomers in Aroclors," *J. Assoc. Offic. Anal. Chem., 55* (4): 746-752 (1973).

[4] R. E. Lea, R. Bramston-Cook, J. Tschida, "Pattern Recognition for Identification and Quantitation of Complex Mixtures in Chromatography", *Anal. Chem. 55*: 626-629 (1983).

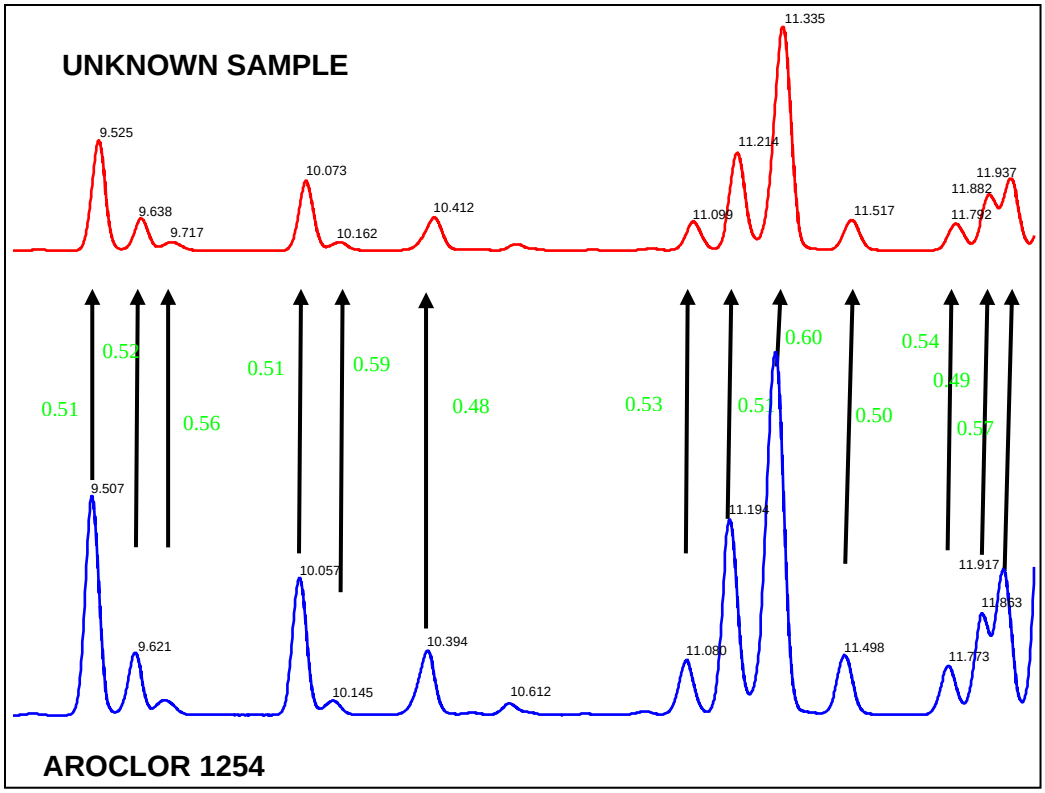


Figure 2. By ratioing peaks that correspond in retention times, a match is confirmed when consistency is demonstrated. For this portion of the chromatogram - 9.3 to 12.0 minutes, the relative standard deviation for the ratios (in green) is 6.7%. This very low deviation exhibits a good match between the two chromatograms. The concentration of the unknown is 54% of the Aroclor 1254 amount – the average of the ratios.

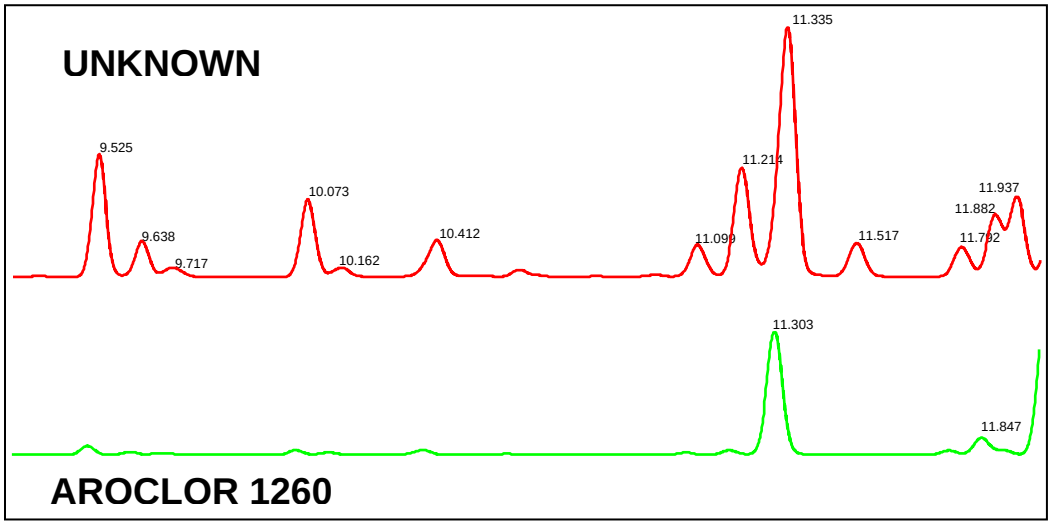


Figure 3. For the same region as Figure 2, Aroclor 1260 matches in retention time with only one peak. The pattern for Aroclor 1260 is obviously not consistent with this portion of the unknown sample's chromatogram. All of the other Aroclor standards exhibit similar non-compliance.

Mixtures of Aroclors are processed by determining that all peaks in a standard are detected in the unknown. Then a moving window is examined across the possible ratios looking for consistent ratios within the window (see Figure 4). Results for mixtures are reported as the average of the ratios from the window that yields the most consistent ratios (within guidelines set up).

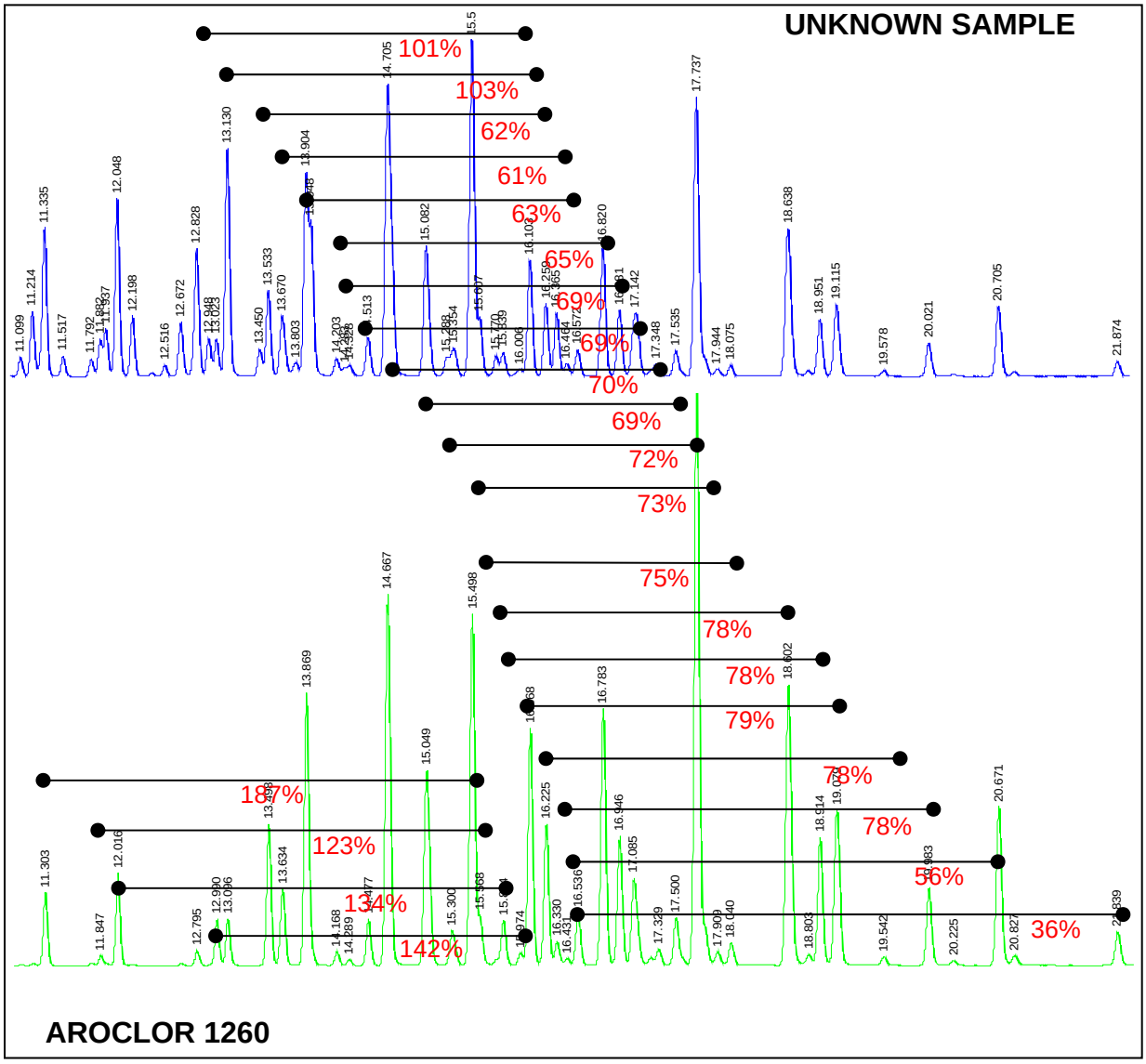


Figure 4. Possible mixtures are detected by moving a window across the chromatogram and looking for a consistency in the ratios within that window. All possible windows are examined for consistent ratios. If the lowest relative standard deviation in the set meets the criteria set in configuration, the match is indicated as "Possible". In the above example, the percentage of standard peaks for mixtures is set to 40% (or 16 peaks) and is indicated by the bar. The lowest deviation is in the last window with a deviation below a criterion of 50%. The concentration becomes the average of the ratios within this window.

Computed statistics indicate the quality of the match; a low standard deviation for the data set indicates a “good” match; “poor” matches yield very inconsistent ratios and high reported standard deviations. Criteria for setting up the matches are preselected by the operator. An example of a report for a positive Aroclor result is shown in Figures 5 and 6.

Major Laboratories
2345 Main Street
Long Beach, California
222/652-6911

PCB PATTERN MATCHING ANALYSIS REPORT

SAMPLE		ANALYSIS	
Sample Name:	Lab #27617	Extraction Date:	08/03/99
Source:	Transformer 42314	Injection Date:	08/03/99 17:27
Activity:	Quarterly Check Sample	Operator:	R B-COOK
Sample Type:	Transformer Oil	Instrument:	PCB Analyzer #6
Collection Date:	08/01/99	Module:	44
Reception Date:	08/03/99	Channel:	Front = ECD
Volume:	10.00	Run Filename:	Lab #27617 8-3-99
Weight:	12.60	Report Date:	08/04/99 9:25

STANDARD NAME	INJ. DATE	OUTCOME	CONC.	%RSD
PCB1026	08/03/99 10:19	< Standard	0.0	
PCB1221	08/03/99 10:50	< Standard	0.0	
PCB1232	08/03/99 11:22	< Standard	0.0	
PCB1242	08/03/99 11:54	< Standard	0.0	
PCB1248	08/03/99 12:24	Failed RSD	0.0	156.2%
PCB1254	08/03/99 12:55	Confirmed	97.9	3.9%
PCB1260	08/03/99 13:24	Failed RSD	0.0	170.9%
PCB1262	08/03/99 13:54	Failed RSD	0.0	169.5%
PCB1268	08/03/99 14:54	Failed RSD	0.0	128.9%

“TOTAL PCB CONCENTRATION FOR LAB #27617 IS 97.9 mg/kg”

“WARNING – TOTAL PCB CONCENTRATION EXCEEDS 50 PPM!”

Matching Parameters:

Number of Standards	9	Retention Time Tolerance Width	0.04min.
Minimum Peaks for Valid Match	20	Retention Time Tolerance Percent	0.30%
Percent of Standard Required	70%	RSD Tolerance	40.00%
Portion of Mixture Required	40%		

Approved by:_____ Date:_____

Figure 5. The front page for the report provides sample information, standards employed, matching results, total PCB concentration, and matching parameters. Additional pages can be optionally printed detailing data employed in the matches.

PCB PATTERN MATCHING ANALYSIS REPORT

(continued)

Computed Data for Matches
Sample Name: Lab #27617
Injection Date: 08/03/99 17:27

Run Filename: Lab #27617 8-3-99
Computed Ratios for Standard: PCB1254
54 of 55 standard peaks were used for matching.

Standard Peak #	Sample Peak #	Retention Time	Conc. (ppm)	Standard Peak #	Sample Peak #	Retention Time	Conc. (ppm)
1	1	8.57	103.3	28	28	13.68	96.6
2	2	8.86	104.0	29	29	13.81	99.7
3	3	9.53	95.0	30	30	13.96	94.4
4	4	9.64	98.5	31	31	14.21	101.6
5	5	9.72	100.2	32	32	14.28	109.0
6	6	10.08	96.6	33	33	14.34	99.3
7	7	10.16	102.2	34	34	14.52	96.5
8	8	10.41	96.5	35	35	14.72	94.3
9	9	10.63	100.6	36	36	15.08	94.7
10	10	11.10	102.1	37	37	15.29	97.6
11	11	11.22	97.5	38	38	15.37	97.5
12	12	11.34	94.1	39	39	15.54	92.8
13	13	11.52	95.4	40	40	15.61	99.2
14	14	11.79	101.1	41	41	15.78	95.6
15	15	11.88	98.5	42	42	15.97	85.6
16	16	11.94	94.2	43	43	16.11	96.9
17	17	12.05	93.5	44	44	16.27	98.4
18	18	12.20	93.7	45	45	16.37	96.7
19	19	12.39	106.4	46	46	16.47	98.8
20	20	12.52	97.8	48	48	16.83	98.4
21	21	12.68	95.5	49	49	16.99	99.0
22	22	12.83	93.9	50	50	17.16	98.6
23	23	12.95	96.1	51	51	17.34	100.2
24	24	13.03	98.6	52	52	17.54	97.8
25	25	13.13	92.8	53	53	17.74	98.8
26	26	13.45	96.9	54	54	18.64	100.2
27	27	13.54	96.4	55	55	20.71	107.0

Relative Standard Deviation = 3.93%

Figure 6. Data involved in the match process can be printed for each standard where sufficient peaks match in retention times. The underlined entries indicate peaks employed in computations for relative standard deviations and for the final concentration.

PCB PATTERN MATCHING CALIBRATION REPORT

Report Date: 8/3/99
Operator: R B-Cook
Workstation: PCB Analyzer
Instrument: PCB Analyzer
Channel: Front = ECD

STANDARD NAME	OUTCOME	STANDARD CONC.	NUMBER OF PEAKS	RUN FILENAME	INJECTION DATE
PCB1221	Pass	100.00	16	pcb1221 8-3-99	08/03/99 10:50
PCB1016	Pass	100.00	26	pcb1016 8-3-99	08/03/99 10:19
PCB1232	Pass	100.00	41	pcb1232 8-3-99	08/03/99 11:22
PCB1242	Pass	100.00	47	pcb1242 8-3-99	08/03/99 11:54
PCB1248	Pass	100.00	43	pcb1248 8-3-99	08/03/99 12:24
PCB1254	Pass	100.00	55	pcb1254 8-3-99	08/03/99 12:55
PCB1260	Pass	100.00	43	pcb1260 8-3-99	08/03/99 13:24
PCB1262	Pass	100.00	44	pcb1262 8-3-99	08/03/99 13:54
PCB1268	Pass	100.00	17	pcb1268 8-3-99	08/03/99 14:54

Approved by:_____ Date: _____

Figure 7. When a complete sequence of standards is run, a special calibration report is created to document standards utilized. An example of such report is shown.

Retention time reproducibility is crucial for the success of this approach. Peaks in samples are matched with corresponding peaks in standards by time. Any shift of retention will cause an incorrect correlation of peaks and improper ratioing, always yielding a negative result even for positive samples. Every effort must be expended to keep retention times consistent. Chromatographic conditions, including column temperature and flow, must remain identical for both standards and samples. Any change in conditions, even subtle changes, will mandate the rerunning of standards with the new conditions prior to examination of unknowns.

Matching abilities are enhanced with a greater number of peaks. Although this matching approach works with packed column chromatography, capillary columns offer greater separation of congeners and an increase in the peak count. Narrow bore capillary columns (0.25 mm ID) are a good compromise between generating a good number of peaks and keeping the analysis time reasonably short. Most Aroclor standards yield over 30 peaks with this type of column. The choice of column phase is not critical, as most commonly used phases will yield acceptable chromatograms for Aroclors.

PCB samples exposed to harsh environmental conditions can lose many of the characteristic peaks due to alterations in the number of chlorines of the biphenyl rings. Historically, these samples required identification of each congener by mass spectrometry; the Aroclor source could not be ascertained. However, with this new approach for matching, a definite pattern can be detected when some of the peaks are excluded by virtue of severe peak overlap from other Aroclors and from coeluting interferences. Figures 8A and 8B show patterns for nine common Aroclors and a pattern obtained from a NIST carp fish tissue. Figure 9 and 10 illustrate recognition of Aroclors 1260 and 1254 patterns in the fish tissue. While every possible Aroclor pattern could be properly detected, often patterns can be discerned only with careful choices of matching parameters. Some experimentation with matching parameters is required to force the pattern matching to equal human cognitive abilities.

Use of surrogate standards for monitoring extraction efficiencies is possible through special treatment of peaks assigned with peak names by Star. Concentrations for surrogates are computed through normal Star calculations. Recovery limits can be defined by the Pattern Matching program for each surrogate and a test outcome against those limits indicated in the final report.

Scalar factors can be employed to make corrections to concentrations measured directly from the pattern matching. Sample weight for transformer oil can be adjusted through a divisor of all results and a dilution factor from the oil processing is adjusted through a multiplier of results - all to convert the final answer into required units of “mg/kg”. As another example, for the fish tissue in Figure 8, the multiplier is employed to correct for a change in the capillary flow ratio and the divisor becomes a correction for a change in injection volume.

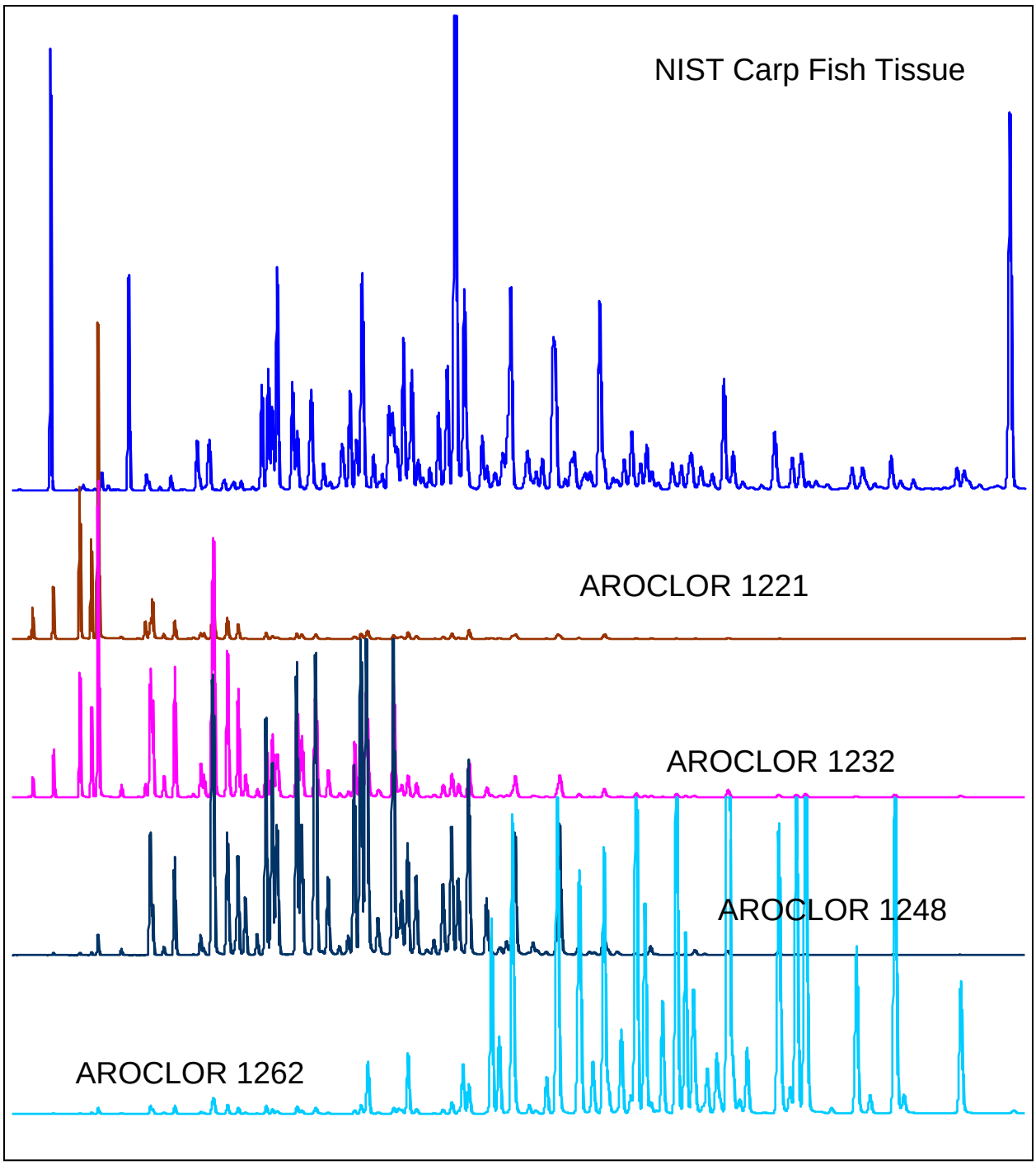


Figure 8A. Each Aroclor generates a unique chromatographic pattern. By ratioing peak areas of the unknown sample with the areas from the corresponding peaks in the Aroclor standard, consistency in the ratios will point to the identification of the Aroclor in the unknown sample, even "environmentally-degraded" samples such as fish tissue. Figures 9 and 10 illustrate the peak matching for two of the Aroclors shown in the set of chromatograms.

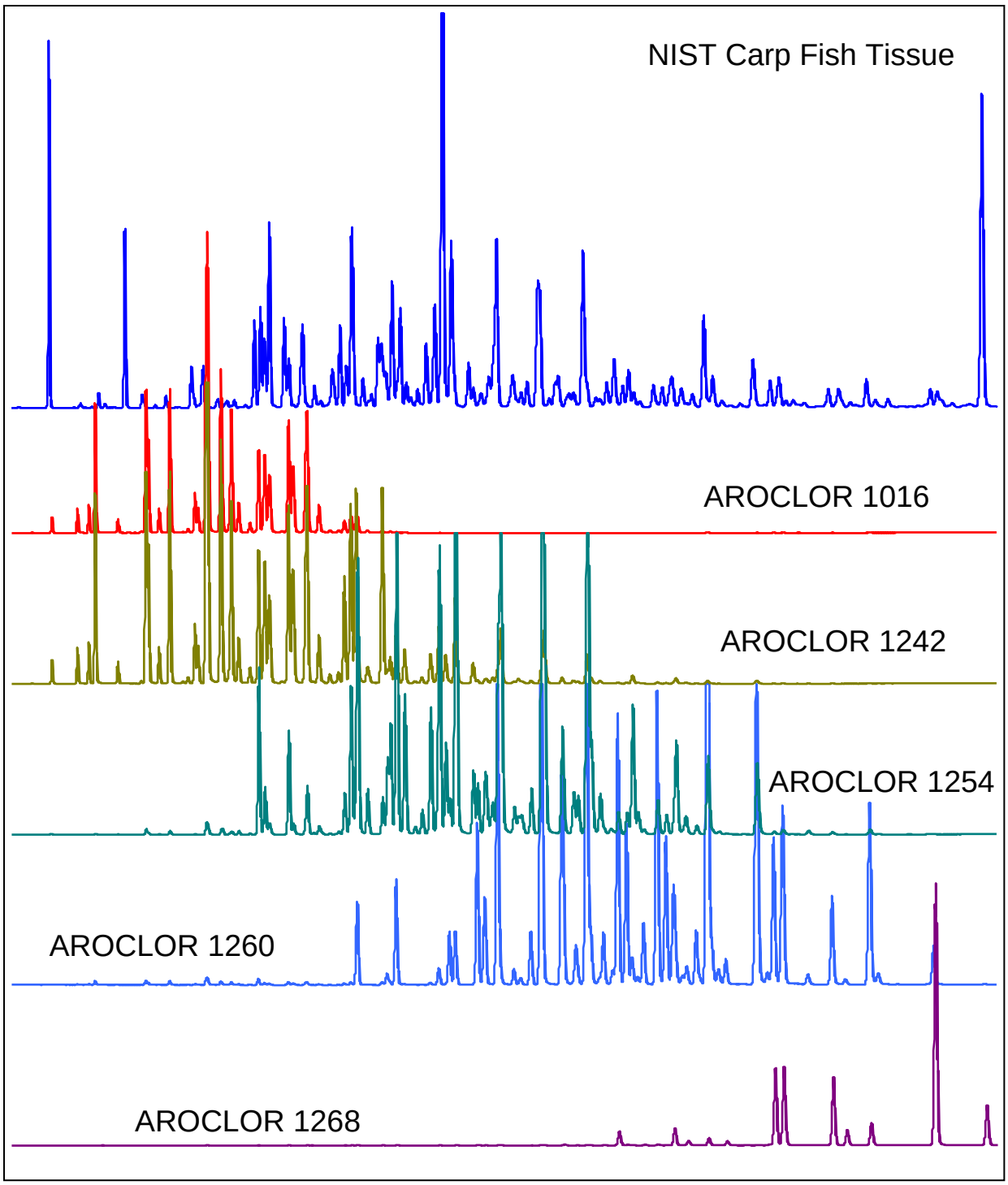


Figure 8B. Additional chromatograms for Aroclor standards that can be employed to match up with peaks in unknown samples are shown.

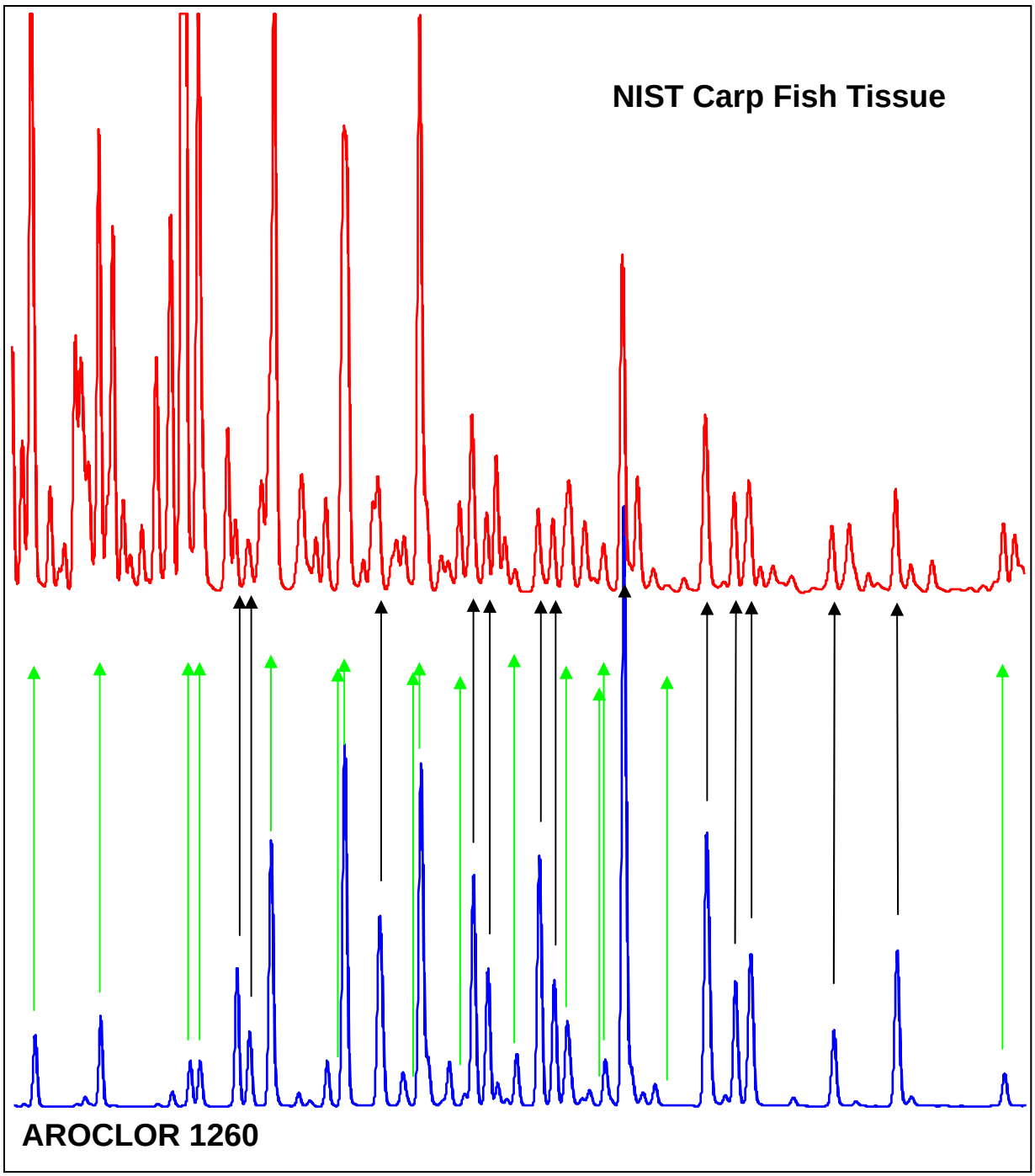


Figure 9. All major peaks in Aroclor 1260 (retention times from 11.3 to 21.8) matched up nicely with peaks detected in a NIST carp fish tissue for the same region. By selecting 13 major peaks (**black** arrows), the recomputation yields an RSD of 21.5%, indicating a good match; and the concentration of Aroclor 1260 becomes 0.69 ppm in the extract solution. Sixteen major peaks (green arrows) are excluded due to severe overlap from other components, but their retention time correspondence provides additional assurance of the match.

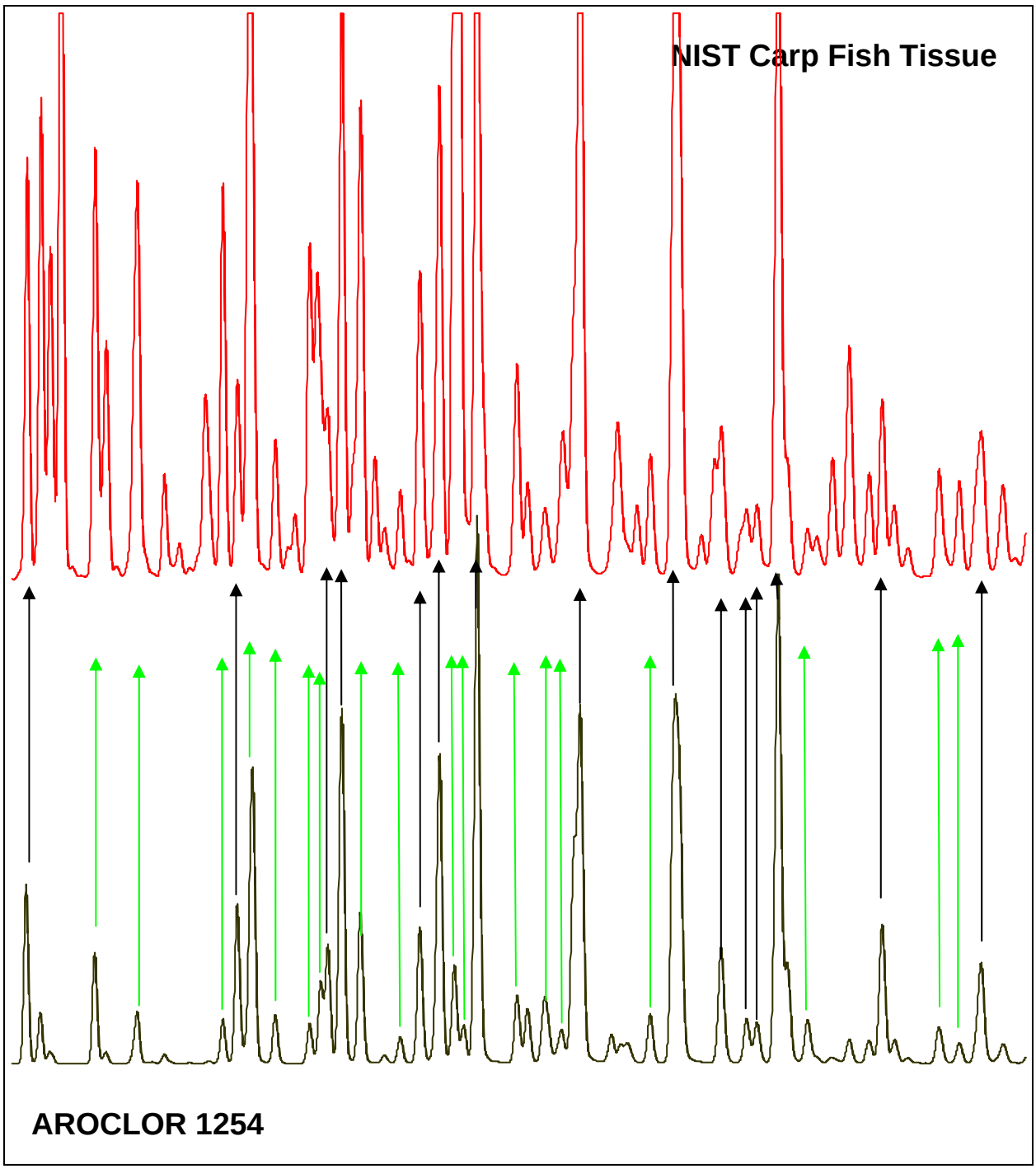


Figure 10. In another portion of the chromatogram for NIST carp fish tissue, Aroclor 1254 (retention times from 9.5 to 17.3) matched up nicely with fish tissue peaks in the same region. By selecting 15 major peaks (**black** arrows), the recomputation yields an RSD of 38.2%, indicating a good match; and the concentration of Aroclor 1254 becomes 1.86 ppm in the extract solution. Nineteen major peaks (green arrows) are excluded due to severe overlap from other components, but their retention time correspondence provides additional assurance of the match.

Advantages of this matching program over other approaches are:

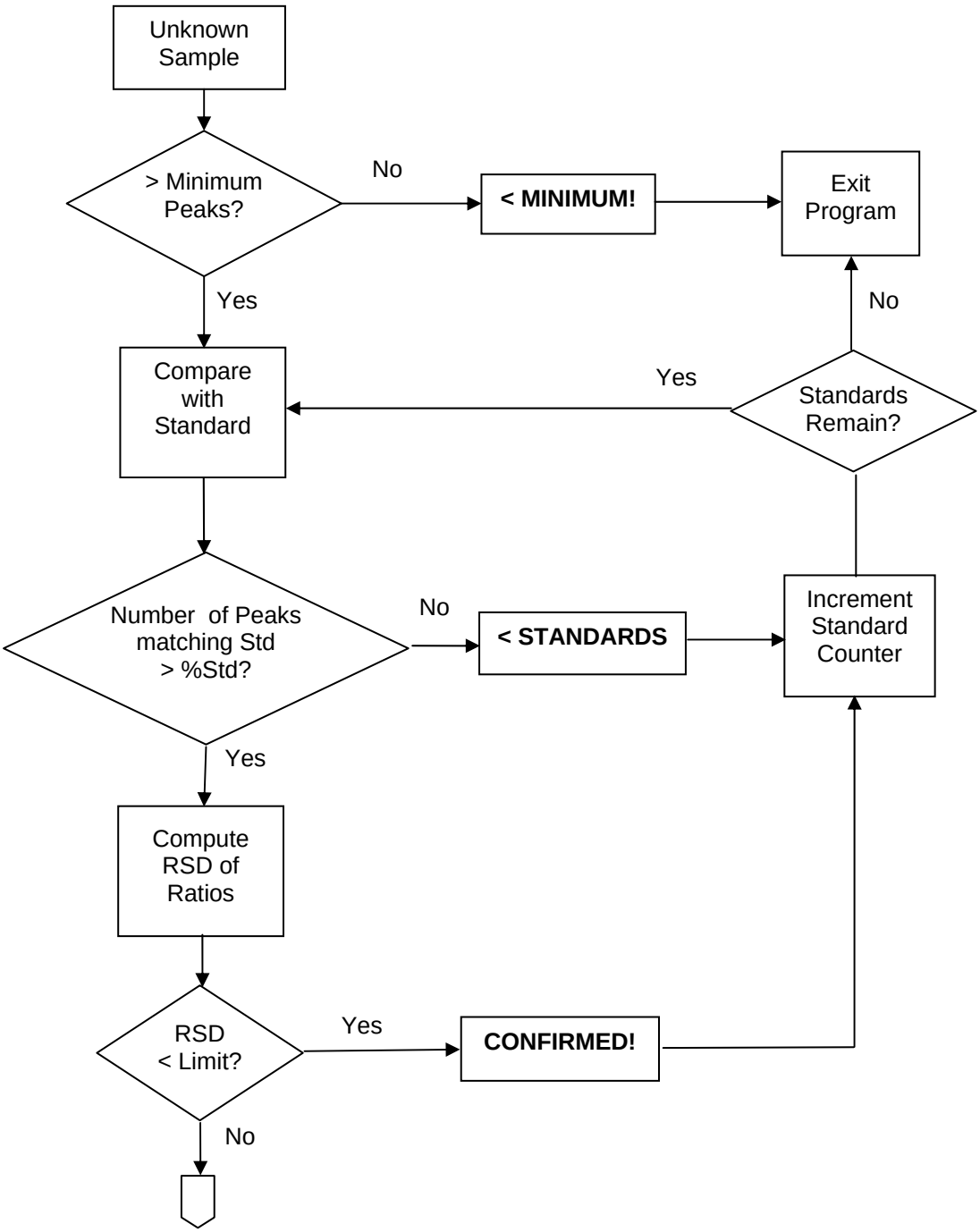
- Automated identification of specific Aroclors
- Direct quantitation of individual Aroclors and possible mixtures of Aroclors
- Aroclor standards selected by the operator and run with unknowns under identical chromatographic conditions
- Chromatography optimized by choices of the operator; no preset conditions mandated
- Applicable to both capillary and packed-column chromatography
- Automatic exclusions of interfering pesticides and other non-“Aroclor” peaks
- Complete operator control of matching parameters and criteria
- Confidence level of match reported
- Matching performed with simple mathematics
- Results can be reviewed and recomputed, if needed, prior to printing hard copies
- Surrogate peaks are reported with their recoveries, and are excluded from the matching process

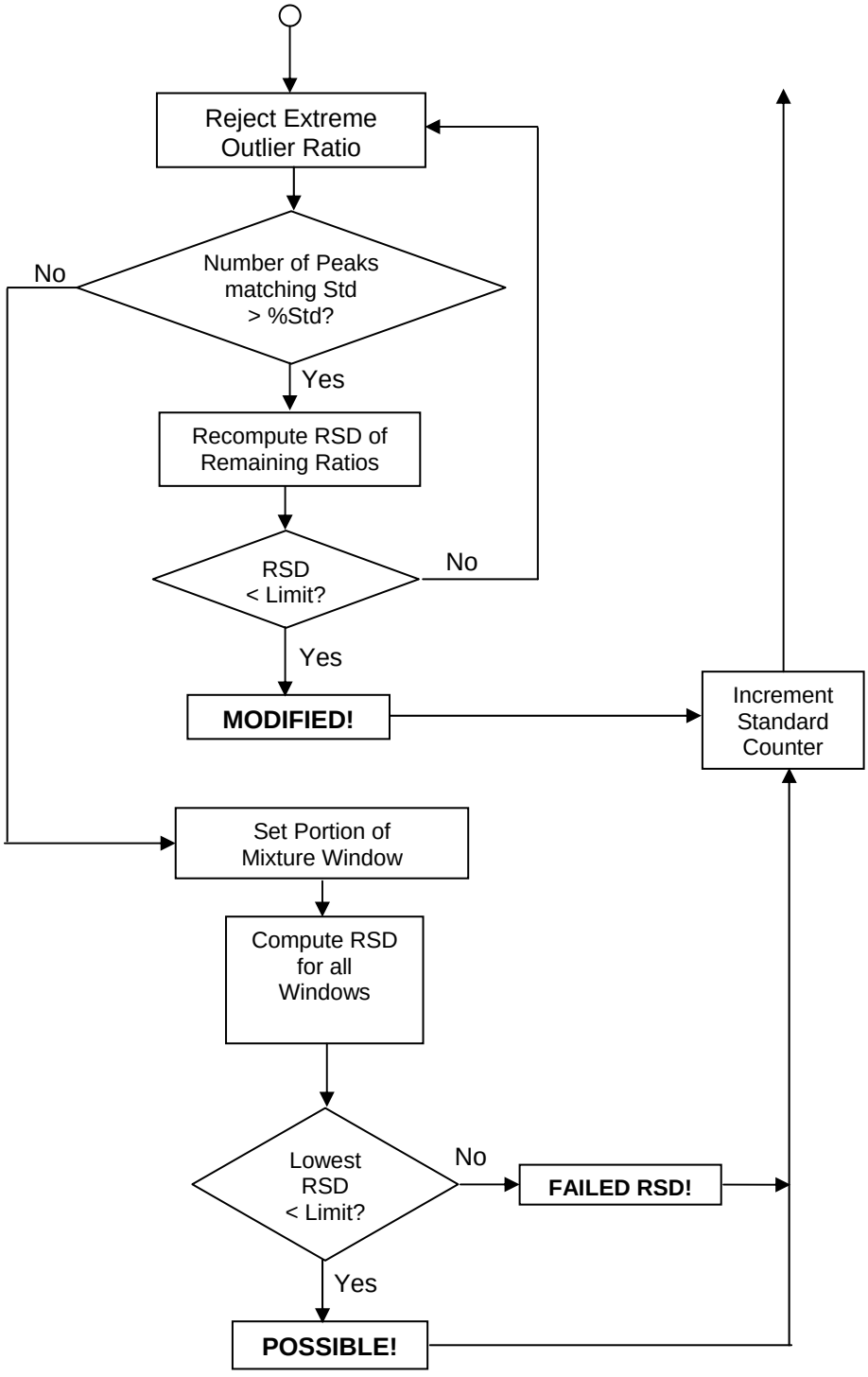
This program is written specifically for the Varian Star Workstation chromatography software, Version 5. Data for matching are gleaned directly from Star Workstation .RUN files and do not require ASCII conversion of reports, as mandated in earlier versions of Star.

Overview of PCB Pattern Matching

Retention times and raw area counts for unknown standards are collected through the gas chromatograph and stored for subsequent comparisons. The expected number of standards must be run before samples can be examined. Then unknowns are examined. If the number of peaks in the unknown is below the user-set threshold, the matching process is terminated for less than minimum peaks with the label “< Minimum”. The next test is comparing the number of peaks found in the unknown with those in the standard; if the number is less than another defined threshold, the match is denied with the label “< Standard”.

A simplified flow diagram for matching decisions is:




Reject Extreme Outlier Ratio
Number of Peaks matching Std > %Std?
No
Yes
Recompute RSD of Remaining Ratios
RSD < Limit?
No
Yes
MODIFIED!
Increment Standard Counter
Set Portion of Mixture Window
Compute RSD for all Windows
Lowest RSD < Limit?
No
FAILED RSD!
Yes
POSSIBLE!

If sufficient number of peaks match up with the standard, the peak areas are ratioed with standard areas by matching retention times. If these ratios are consistent with a given Aroclor, then a match is "confirmed". If the number of peaks in the unknown is significantly less than the number in the standard, a match is not possible. If peaks in the unknown match with standards in retention times, but the ratios are very inconsistent, possible interfering pesticides or other non-"Arolcor" are excluded by mathematically removing the extreme high and low deviates until an adequate number of peaks still remain. If the remaining peaks show consistency, then a match is indicated as "Modified", with peak exclusions.

If peaks in the unknown match with standards in retention times, but the ratios stay inconsistent, even after the exclusions for pesticides, the variations could be attributed to an overlap of several Aroclors. To sort out mixtures, a user-specified percentage of the total standard peaks becomes a window to look for consistency in the ratios. The program examines all possible windows in the chromatogram and computes their relative standard deviation. If the lowest ratio set meets the standard deviation criteria, the mixture is listed as "Possible". If consistency is still not detected, the results are labeled as "Failed RSD". Even if a match is not made through the automatic sequence, the operator can manually select peak ratios that appear consistent to recompute a possible match. This match is listed as "Recalculated".

Possible outcomes for matches are:

- **Confirmed** – Peak times for the sample match up with a standard and peak ratios are consistent. The match was perfect!
- **Modified** - Peak times for the sample match up with a standard and peak ratios are consistent after outlying peaks are rejected, but the required number of peaks for a match are still maintained. Interfering peaks from pesticides or other non-PCB peaks have been rejected and a pattern match still was identified.
- **Possible** – Peak times for the sample match up with a standard, but peak ratios fail to pass the consistency test. However, a portion of the match demonstrates that a mixture is possible with a consistent ratio found in that portion. Results should be reviewed to confirm that a mixture of several Aroclors is possible.
- **Failed RSD** – Peak times for the sample match up with a standard, but no consistency in the ratios is detected. Results can be rechecked through manual editing of results through View Reports; a possible PCB match is likely, but failed all of the automatic tests.
- **< Standards** – Number of peaks found in the sample is less than the required percentage of peaks in the standard. A match with a standard is not permitted when only a few peaks match in retention times.
- **< Minimum** – Number of peaks found in the sample is less than the threshold set by the user. With minimal peaks found, no match is possible.
- **Recalculated** – Results were achieved by manually selecting peaks through offline operations. Matching criteria must still be met before results are acceptable.

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