Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking

Application

Gas Chromatography

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Abstract

Complete development of a gas chromatographic method often involves a significant amount of effort. Once a method is completed, retention time locking (RTL) can be used to implement the method and to obtain the same retention times on multiple systems. This application note describes how to use method translation combined with RTL to implement precise time-scaled versions of a method on multiple instrument types. This allows the original method to be re-used with minimal effort, while optimizing the method for a given sample type or instrument setup. In this way, the utility of the original method is extended greatly, increasing the payback on the investment in its development and optimizing its use for specific analyses. In this note, the Agilent RTL Pesticide Library method is used as an example. The steps involved in precise time-scaling of the method to different speeds, detectors, and columns are presented.

Key Words

Pesticides, GC, GC-AED, retention time locking, RTL, method translation, scalable RT libraries

Introduction

Interest in the analysis of pesticide residues has been increasing recently, in part due to the discovery that some of these compounds act as endocrine disrupters. Agilent Technologies has responded to the need for rapid, accurate, and comprehensive screening analysis for pesticides by developing a method to screen for 567 pesticides and suspected endocrine disrupters. The method uses element- selective detection and a retention time locked library of retention times to find and identify pesticides in a sample.[1]

In the method, sample extracts are run with element-selective detection using a prescribed set of chromatographic conditions and with the column retention time locked to the retention times in a table. If any peaks containing heteroatoms are observed, the section of the table corresponding to a small time window around the observed peak is searched. The time search results are further sorted using the observed element content of the peak. The combination of time and element content narrows rapidly the possible compounds that could have produced the heteroatom response to a few pesticides.

The element-selective detection is done with either gas chromatography-atomic emission detection (GC-AED), which can screen for all the individual elements found in pesticides, or with a combination of other selective detectors like the electron capture detector (ECD), the nitrogen-phosphorus detector (NPD), the flame photometric detector (FPD), or the electrolytic conductivity detector (ELCD).

The GC-AED technique can also be used to calculate element ratios and to quantitate unknown peaks that are detected because of its equimolar element response factors. The measured element ratios can be used to further distinguish between possible identities of detected heteroatomic compounds, often resulting in a single entry as the likely identity of a given peak. With compound-independent calibration, the amount of the unknown can be calculated using element response factors generated with a different standard compound.

Once the element-selective screen is completed, samples that contain any suspect compounds are run on a GC with mass spectral detection (GC-MS) system that is retention time locked to the pesticide method, thus having the same retention times as the element-selective detectors. Using the possible identities generated from the element screen, the GC-MS data is evaluated to decide which (if any) of the possible identities for suspect peaks is correct. The confirmation process is simplified greatly because the element screen usually yields only a few possibilities and because the retention time in the GC-MS run is accurately known. In practice, extracted ion chromatograms for characteristic ions of each possible compound are used to determine the identity of suspect compounds.

This screening method minimizes false negatives, even in dirty samples, by using element-selectivity and time in the initial screen. With element-selective detection, all compounds containing chlorine, phosphorus, nitrogen, etc. are detected. Even if a detected heteroatomic compound is not in the table, its presence is known, and it can be marked for further GC-MS evaluation. By using GC-MS for confirmation, false positives are also minimized.

The RTL Pesticide Library method is a good example of a method in which a substantial investment of time and material has been made. As with many methods intended for use in multiple laboratories, it would be desirable to be able to scale the method for use in different situations of sample type and instrument setup. Because the method relies on the measured retention times of 567 compounds, it would be impractical to re-measure all the retention times whenever the method is modified, for example, to increase its speed.

Method translation[2–4] is a calculation technique developed at Agilent Technologies that allows a capillary column GC method to be translated to different chromatographic conditions. The technique calculates the required changes in inlet pressure and oven temperature ramp rates and hold times required to maintain peak elution order identical to that of a reference method. In this way, the speed of an analysis can be scaled predictably to accommodate the needs of a specific sample or instrument type.

The inlet pressure calculated for the new version of a method by the method translation software is based on the assumed or nominal dimensions of the column. As such, the calculated inlet pressure will provide a close, but not exact, match to the desired scaled retention times. To match precisely the retention times of the scaled method to the desired scale factor, the new method must be retention time locked. Retention time locking[3] (RTL) is a technique developed by Agilent Technologies whereby the inlet pressure required to match retention times precisely is calculated from a calibration curve of inlet pressure versus retention time.

Using method translation followed by RTL allows a method to be scaled by a precisely known factor. Once the chromatography has been scaled, a retention time table, such as the RTL Pesticide Library, can then be scaled by the same factor, resulting in a new library whose retention times match those of the scaled method precisely.

The steps required to scale the method are:

1. Determine the desired scale factor for the new method.
2. Use the method translation software[4] to calculate the inlet pressure and oven temperature adjustments to obtain the desired scaling of the method. The scale factor is the “speed gain” value reported in the method translation software. Make sure that the new method parameters are consistent with the hardware capabilities of where the new method will be used.
3. Perform the RTL calibration runs for the new method. Alternatively, the method translation software can be used to calculate the RTL calibration points for the new method using those from the original method.
4. Retention time lock the new method using the locking reference standard from the original method. The new method should be locked to the original reference standard retention time divided by the scale factor.
5. Export the retention time table as a text file using the EXPORT function in the RTL SEARCH menu of the RTL ChemStation software.
6. Divide the retention times in the table by the scale factor in a spreadsheet program like Microsoft® Excel™.
7. Re-import the new, scaled table.
8. Run a representative test mixture to validate the scaled method.

Several examples of scaling the HP RTL Pesticide Library are presented below.

Experimental

All data were collected on Agilent 6890 Series GC systems. All systems were equipped with:

- Electronic pneumatics control (EPC)

- Split/splitless inlet
- Automatic liquid sampler

The GC-AED system also included an Agilent G2350A atomic emission detector with GC-AED ChemStation software (rev B.00.00) for Microsoft® Windows NT®.

The GC-micro-ECD system was controlled by Agilent GC ChemStation software (rev A.05.04). Both the GC-AED and the GC-micro-ECD ChemStations contained RTL software for GC ChemStation (G2080AA) and the Retention Time Locking Pesticide Library for GC ChemStation (G2081AA).

The GC-MS system (G1723A) used consisted of an 6890 Series GC equipped with an Agilent 5973 mass selective detector (MSD). The process for retention time locking the GC-MS system is described in reference 2.

All systems except the micro-ECD instrument used 30 m × 0.25 mm id × 0.25 µm HP-5MS columns (part no. 19091S-433). The Agilent micro-ECD instrument used 10 m × 0.1 mm id × 0.1 µm HP-5 column (part no. 19091J-141).

RTL measurements were made with a solution of dichlorvos, methyl chlorpyrifos, and mirex, each at 10-ppm concentration in acetone. All injections were 1-µL splitless, except for the micro-ECD experiments, which were 1-µL split 100:1. In all methods, inlets were operated at 250 °C and detectors at 300 °C.

Method translation requires inlets to be run in constant pressure mode to obtain precise scaling of retention times. Thus, all methods discussed in the note were run in this mode.

Results and Discussion

Locking GC-MS with Other GC Detectors

When using selective GC detectors in conjunction with GC-MS, one problem that is encountered is knowing the relationship between retention times on the selective detector and that of the GC-MS. In GC-MS, the outlet pressure of the column is vacuum, while with most other GC detectors, the outlet pressure of the column is at or near atmospheric pressure. This difference in outlet pressures results in large differences in retention time between GC with MS detection and GC with other detectors. Comparison of GC-FID, a general detector, with GC-MS is reasonably straightforward, because the total ion chromatogram (TIC) of the GC-MS system has similar response to the FID. Retention times on the GC-MS system corresponding to those on the GC-FID can be determined by looking for similar patterns of response. With selective detectors, this is much more difficult because the response patterns from selective detectors usually do not resemble the TIC. For this reason, matching the retention times of selective detectors precisely with the GC-MS system simplifies data analysis greatly.

In this first example of scaling the RTL Pesticide Library, the method will be scaled from the GC-AED method to the GC-MS method. In this case, the desired scale factor is exactly 1, that is, the GC-MS retention times are desired to be exactly the same as those of the GC-AED. The first step is to use the method translation software to determine the GC conditions to use for GC-MS.

Figure 1 shows the method translation software. The original method conditions for the GC-AED pesticide method are entered in the column labeled “Original Method.” The column dimensions, carrier gas type, inlet pressure, outlet pressure, ambient pressure, and oven temperature program are entered here. Note that the inlet pressure is in psi (gauge), while the outlet pressure and ambient pressure are psi (absolute). The original method here is being used on a GC-AED system, so the outlet pressure is entered as atmospheric pressure plus 1.5 psi, the operating pressure of the GC-AED.

The “Criterion” parameter is set to “None,” which allows the user to select a specific value of “speed gain” by adjusting the value of hold-up time for the translated method (see figure 1). In the column labeled “Translated Method,” the parameters of column dimensions, carrier gas type, outlet pressure, and ambient pressure for the GC-MS method are entered. Note that the inlet pressure and oven program are not entered; they are calculated by the program. To set the speed gain to a desired value, take the calculated value of hold-up time in the first column (0.996060 minute) and divide it by the scale factor. Because in this case the desired scale factor (“speed gain”) is 1, the same hold-up time for both the GC-AED and the GC-MS methods is required. Clicking the radio button next to the hold-up time in the “Translated Method” column will do this automatically.

The method translation indicates that to obtain the same retention times on the GC-MS system as on the GC-AED, use all the same method parameters

except inlet pressure. Instead of using 27.6 psi as is used on the GC-AED, method translation calculates that 17.93 psi on the GC-MS system will result in matching retention times. As mentioned above, this inlet pressure is calculated on the assumed dimensions of the column in the GC-MS system. To get the retention times to match precisely, RTL[3] is used.

To retention time lock the GC-MS method to the GC-AED method in this example, it is necessary to construct an RTL calibration file for the GC-MS system. Construction of this file only needs to be done once. All subsequent users of the GC-MS method will then be able to use this calibration file for a similarly configured GC-MS instrument.

The RTL calibration file is constructed by running five calibration runs of the target compound, in this case methyl chlorpyrifos, at five different inlet pressures. The runs are made at conditions identical to the nominal method except that four of the runs are made at different pressures. The pressures used are typically:

- Target pressure – 20%
- Target pressure – 10%
- Target pressure (nominal method pressure)
- Target pressure + 10%
- Target pressure + 20%

The retention time of the target compound is determined for each run. The resulting set of five pressures and corresponding retention times is then entered in the RTL calibration dialog box for the method and saved with the method.

To lock the method on the GC-MS setup, the target compound is run at

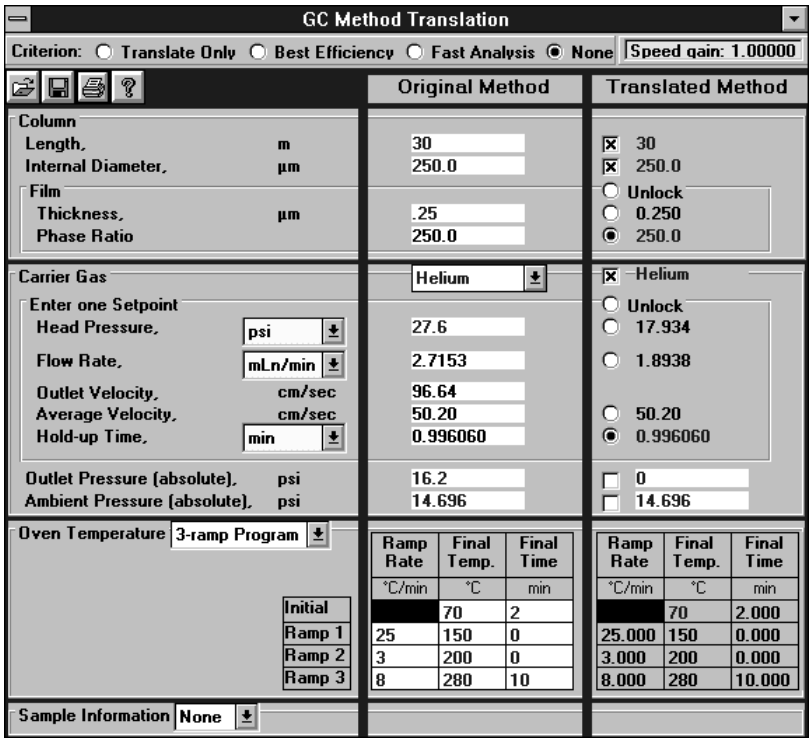

Figure 1. Method translation software showing scaling HP RTL Pesticide Method from GC-AED conditions to GC-MS with a scale factor of 1.

the nominal method pressure, and the retention time is observed. The pressure and resulting retention time are then entered into the "(Re)Lock New Column" menu item of the RTL software to calculate the correct pressure for obtaining locked retention times.

Normally, the RTL calibration for a new method is determined by actually making the five calibration runs. In the current example, methyl chlorpyrifos would be run at:

- 17.93 psi – 20% = 14.34 psi
- 17.93 psi – 10% = 16.14 psi
- 17.93 psi (nominal method pressure)
- 17.93 psi + 10% = 19.72 psi
- 17.93 psi + 20% = 21.56 psi

However, because the new GC-MS method is scaled from an existing GC-AED method that already has RTL calibration data, method translation can be used to calculate the new RTL calibration points. This is useful when you want to try a scaled method rapidly and save the time required in making the five runs. (Note: For methods that will be used extensively, the five-runs approach may provide a somewhat better calibration. It is recommended that for these methods, the standard calibration be performed.)

To calculate the five RTL calibration pairs of pressure and retention time for the GC-MS method from those of the GC-AED method:

- Take the inlet pressure used for each original GC-AED RTL calibration run, and enter it into the method translation software for the inlet pressure of the original method. Make sure the hold-up times are locked, giving a "speed gain" of 1.

- The inlet pressure calculated in the "Translated Method" column will now change to a new value, corresponding to the pressure that would be obtained if the calibration run were made on a GC-MS system. This pressure is used with the retention time obtained for the corresponding GC-AED calibration run as a calibration point for the GC-MS method.

When all five points have been calculated in this way, they are entered into the RTL calibration dialog box for the GC-MS method and saved with the method. Table 1 lists the original RTL calibration pressures and times with the calculated pressures and times for the GC-MS method.

To test the accuracy of using a predicted RTL calibration file for GC-MS, a real calibration set was measured on the GC-MS system. The data is shown in the first two columns of table 2. (Note: The calibration points are spaced ~ 5% apart in pressure instead of the typical 10%.) A GC-MS RTL calibration file was constructed with these measured points. For each point, the locking pressure required to lock the method was calculated and is shown in column 3 of table 2.

The locking pressure is the pressure determined by the RTL software that would make methyl chlorpyifos have a retention time of 16.596 minutes. This is determined by entering the pressure and retention time for each point into the "(Re)Lock New Column" menu item of the RTL software. If the calibration is done correctly, the locking pressures determined from each point should be very similar, as they are in column 3 of table 2.

Column 4 of table 2 shows the locking pressures for the same set of runs but determined using the GC-MS RTL calibration points calculated using method translation. The calculated data provide locking pressures that agree well with those based on measured data. The range in locking pressures pressure is only from 17.72 to 17.75 psi. This range of 0.03 psi corresponds to only about a 0.006-minute range in the retention time of methyl chlorpyrifos.

Figure 2 shows the locked chromatograms from a three-component mixture run on GC-AED and GC-MS systems. As can be seen, the retention times are well matched between the two methods.

The RTL Pesticide Library contains the retention times of the 567 pesticides measured with GC-FID. The values measured with the FID would be the same observed with any detector that is operated at or near atmospheric pressure. Because retention time matching is critical in this application, the retention times for all the compounds in the table were also measured on the GC-MS system after scaling as described here. Figure 3 is a plot of the difference between the retention times measured on the GC-FID and the GC-MS systems. The plot shows the retention times match well within ± 0.1 minute out to 30 minutes. A few compounds at the end deviate outside this window, with one compound 0.2-minute different. The

Table 1. RTL Calibration Points from Original GC-AED Method and Calculated Points for GC-MS

GC-AED RTL Calibration		GC-MS RTL Calibration	
Pressure (psi)	Ret Time (min)	Calculated Pressure (psi)	Calculated Ret Time (min)
33.1	15.346	24.27	15.346
30.4	15.919	21.18	15.919
27.6	16.578	17.934	16.578
24.8	17.338	14.654	17.338
22.1	18.242	11.449	18.242

Table 2. Comparison of Locking Pressures Calculated Using Measured and Predicted GC-MS RTL Calibration Data

GC-MS Locking Runs		Locking Pressures	
Measured GC-MS RTL Cal Points		Using Measured RTL Cal Points	Using Calculated RTL Cal Points
Pressure (psi)	Ret Time (min)	Pressure (psi)	Pressure (psi)
20	16.127	17.73	17.75
19	16.326	17.72	17.73
18	16.536	17.72	17.72
17	16.760	17.74	17.74
16	16.988	17.72	17.74

deviation is clearly largest in the isothermal hold region, which starts at 31.87 minutes. This effect is seen with GC-MS, but not with scaling to other atmospheric pressure detectors. While the cause is not yet clearly understood, it appears related to the vacuum outlet pressure of the GC-MS column. Although this level of matching is very good, the table includes both the GC-FID and GC-MS retention times so that smaller time windows can be used in searching unknowns.

Locking GC-AED with Other GC Detectors

When the method translation step is done to scale the GC-AED method to other atmospheric pressure detectors, the only different parameter to enter is the outlet pressure. The outlet pressure for the GC-AED method is 16.2 psi and that for the others is 14.696 psi. The method translation calculates that the nominal GC-AED inlet pressure of 27.6 psi would be changed to 26.29 psi for the other atmospheric detectors. This difference (<5%) is so small that it can be neglected, because corrections in this range are compensated easily by the retention time locking step. Thus, the method conditions and RTL calibration points used with GC-AED are interchangeable with FID, NPD, ECD, FPD, and other atmospheric detector methods.

Note that this would not always be the case. If for example, a method is being scaled that uses a very low inlet pressure, the 1.5-psi difference in outlet pressure could become significant. It is best to check the method with method translation and see if the inlet pressure will change by >10%. If it does, it would be advisable to collect (or translate) a new RTL calibration centered around the translated nominal inlet pressure.

Gaining Speed in the Same Instrument Setup

In the analysis of pesticide residues in food, there are usually only a few compounds encountered in any one sample. Because the screening method uses selective detectors, it makes sense to consider trading speed for chromatographic resolution. Selective detectors respond to only those compounds containing a specific heteroatom(s), and the chromatography only needs to resolve those compounds from each other, not from every other compound in the matrix. This approach can save a significant amount of analysis time.

In this example of scaling the RTL Pesticide Library, the method will be increased in speed at the expense of chromatographic resolution. The first consideration is by what factor to increase the speed. The method translation software is useful for determining this. A candidate speed gain, in this example threefold, is entered into the method translation software. The resulting inlet pressure and oven temperature ramp rates are then inspected to see if the instrument on

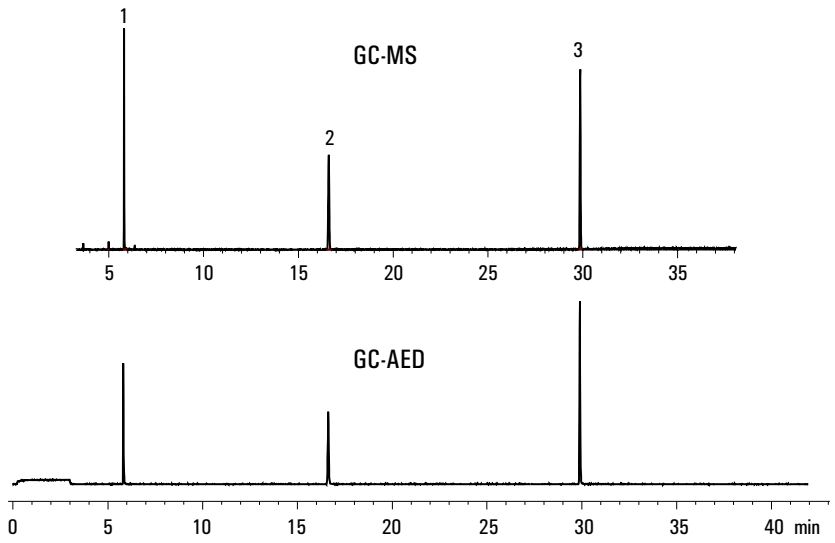

Figure 2. GC-AED chlorine and GC-MS TIC chromatograms of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.

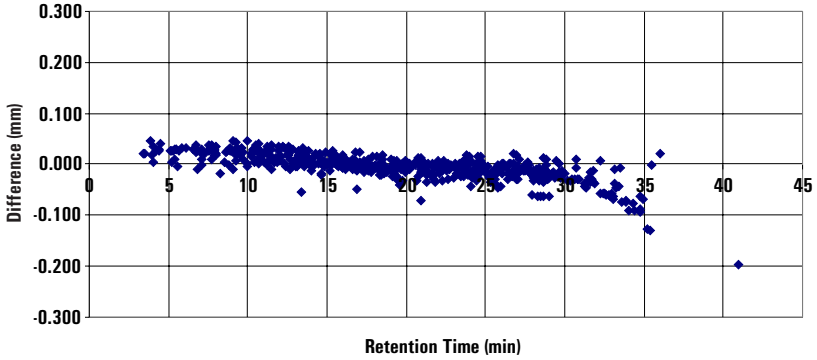

Figure 3. Difference plot of GC-MS and GC-FID retention times in RTL Pesticide Library.

which the new method will be run is compatible with those parameters.

Figure 4 shows the method translation software with the data entered for a speed gain of 3. Note that columns for "Original Method" and "Translated Method" are set up as in the previous example with two exceptions. Because the scaling is from GC-AED to GC-AED, the outlet pressure in both columns is entered as 16.2 psi. The second and most significant difference is the holdup time. The desired "speed gain" is 3.

To set the speed gain, the calculated value of hold-up time in the first column (0.996060 minute) is divided by exactly 3. This value (0.33202 minute) is entered for the hold-up time in the second column. This will force the speed gain to exactly 3.

The inlet pressure and oven temperature ramp for the new threefold speed method are now calculated. The calculated inlet pressure is 87.862 psi, which is compatible with the EPC module on the current system (maximum 100 psi). Note that the helium source supplying the GC must be capable of reaching 100 psi of helium. An optional 150-psi EPC module is available for the HP 6890 GC to provide additional inlet pressure, if necessary.

The oven temperature program calculated for the new method has the first ramp listed as 75 °C/min. This ramp rate is compatible with the 240-V oven option on the current instrument but would not work with a 120-V oven, which is limited to about 50 °C/min in this temperature range. With a 120-V oven, the speed gain would be limited to about 2.

The next step is to calculate the RTL calibration points from the original GC-AED method. This is done by the same process as shown in the GC-MS scaling above. In this case, when one of the original method RTL calibration pressures is entered, the resulting holdup time must be divided by 3 and entered for the holdup time in the "Translated Method" column. This will force the "speed gain" back to 3. The resulting inlet pressure is then paired with the retention time of the corresponding original GC-AED calibration run, but divided by 3 as a calibration point for the new method.

Table 3 shows the RTL calibration points from the original GC-AED method and calculated points for the threefold speed gain (3×) method.

When the calibration data is entered into the RTL calibration dialog box, the target time for methyl chlorpyrifos is entered as 5.532 minutes, which is 16.596 minutes divided by 3.

Table 4 compares the locking pressures determined with measured and with calculated RTL calibration points. As in the above GC-MS example, the range of the locking pressures from the calculated data is only 0.11 psi (87.88 to 87.99), which corresponds to ~ 0.003 minute.

Figure 5 compares the chromatograms of the RTL locking mixture from both the original and the 3× scaled methods. Note that while the chromatographic resolution is reduced, the speed is increased by a factor of 3.

Figure 6 shows a plot of the difference between the RTL Pesticide Library retention times, divided by 3, and those of the 3× method. The data were taken with a 36-component subset of the library. The plot shows the retention times match well within ± 0.05 minute for all compounds, even

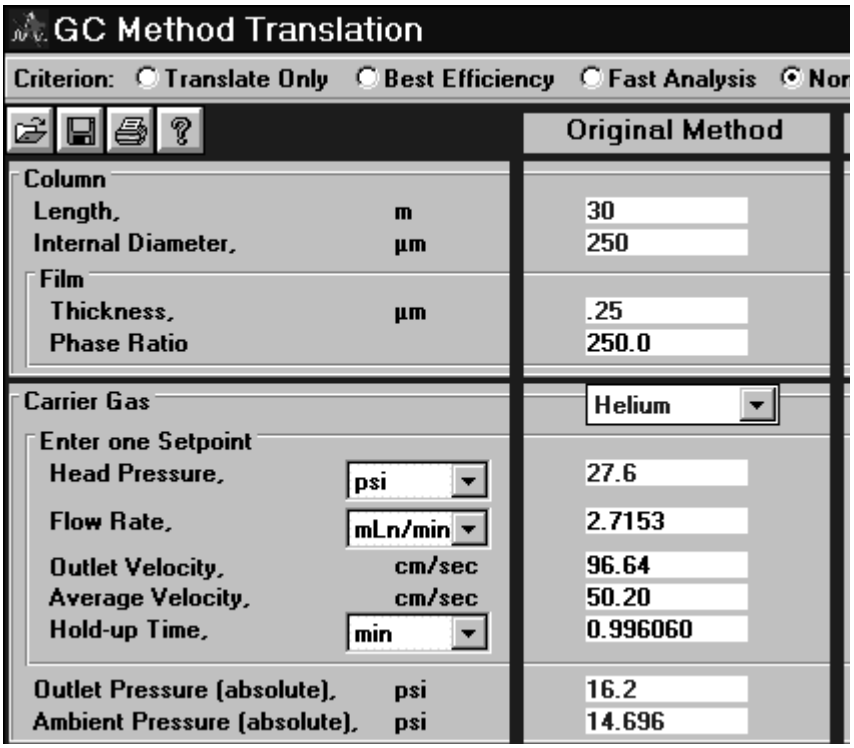

Figure 4. Method translation software showing scaling RTL Pesticide method scaled to threefold faster method.

those in the 3.3-minute hold time at the end of the run.

Gaining Speed with a Small-Bore Column

In the previous example, speed was gained at the expense of resolution. In this example, speed will be gained while maintaining most of the resolution but sacrificing capacity. This is done by scaling the original method to a 0.1-mm id column.

In scaling to columns of a different diameter, there are two important considerations that must be obeyed to obtain precise matching to a library or reference method. The first is that the stationary phase composition must be the same as that used in the original method. The second is that the phase ratio of the column being scaled to must be the same as that of the reference method. Columns of the same phase ratio have the same ratio of inner diameter to film thickness. Because the reference method was developed on a column with 0.25 mm id × 0.25 µm film thickness, scaling to a 0.1-mm id column will require a 0.1-µm film thickness. A 10-m column of these dimensions was chosen for this example.

The micro-ECD for the 6890 GC is extremely sensitive, with detection limits in the low femtogram range for polyhalogenated pesticides. These detection limits are so low that it is reasonable to consider using split mode for a rapid screening method. Using split mode with a split ratio of 100 still gives a detection limits in the range of a few picograms. The split is also more compatible with the relatively low capacity of the column.

Table 3. RTL Calibration Points from Original GC-AED Method and Calculated Points for Threefold Speed Gain (3×) Method

GC-AED RTL Calibration		3x GC-AED RTL Calibration	
Pressure (psi)	Ret Time (min)	Calculated Pressure (psi)	Calculated Ret Time (min)
33.1	15.346	106.21	5.115
30.4	15.919	97.23	5.306
27.6	16.578	87.86	5.526
24.8	17.338	78.44	5.779
22.1	18.242	69.31	6.081

Table 4. Comparison of Locking Pressures Calculated Using Measured and Predicted 3× GC-AED RTL Calibration Data

3x GC-AED Locking Runs		Locking Pressures	
Measured 3x GC-AED RTL Cal Points		Using Measured RTL Cal Points	Using Calculated RTL Cal Points
Pressure (psi)	Ret Time (min)	Pressure (psi)	Pressure (psi)
97	5.319	87.99	87.99
92	5.433	87.94	87.95
87	5.557	87.99	87.99
82	5.689	87.99	87.96
77	5.832	87.97	87.88

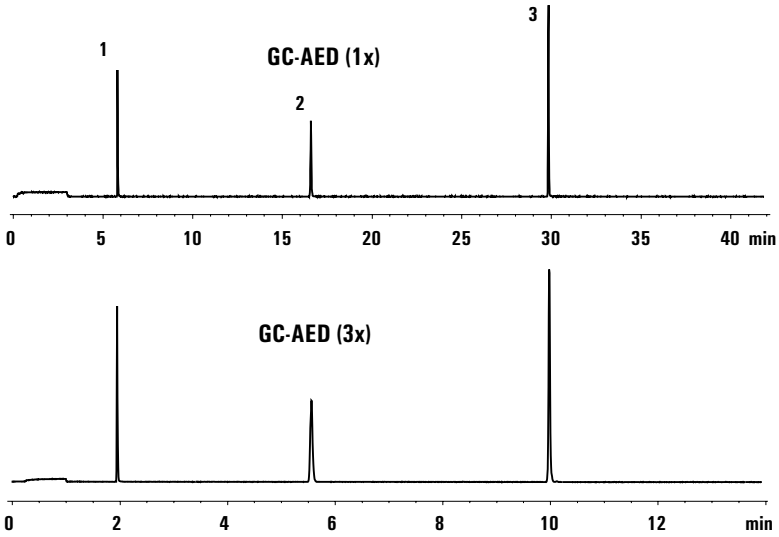

Figure 5. Chlorine chromatograms from original and 3x GC-AED methods of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.

Figure 7 shows the method translation from the GC-AED method to the 0.1-mm id column with a scale factor of 3. A speed gain of 3 was again chosen based on oven and inlet limitations as described above. The same scaling process as used above is followed.

The RTL calibration points for the new 3× 0.1-mm micro-ECD method were both calculated with method translation and measured. Table 5 shows the calculated values.

When the locking pressures from the measured and calculated values were examined, the calculated values provided much poorer predictions of locking pressure than expected. The pressure required to actually lock the column was confirmed to be 65.95 psi, as predicted by the measured RTL calibration data. Method translation had predicted the inlet pressure would be 58.514 psi for an assumed 10-m column length. Because the actual locking pressure was noticeably higher, this suggests that the actual column length was longer and/or the column diameter was smaller and/or the film thickness larger than the assumed values.

As an experiment, it was assumed that the problem was in the assumed length of the column used in calculating the RTL calibration points. The column length entry for the 0.1-mm column was iteratively adjusted until the calculated inlet pressure matched the actual locking pressure, 65.95 psi. This resulted in a calculated column length of 10.5622 m. A new set of calculated RTL calibration points were calculated using 10.5622 m as the length of the 0.1-mm column. The results are shown in table 6.

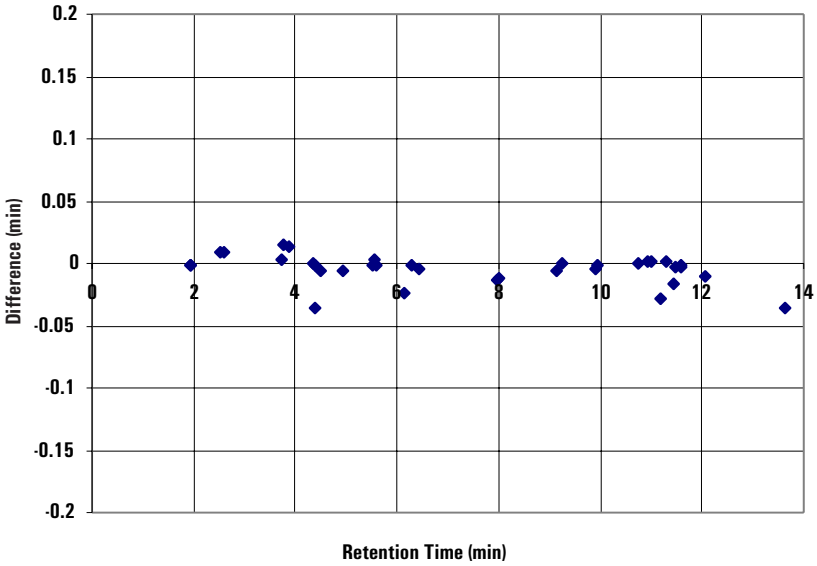

Figure 6. Difference plot of RTL Pesticide Library (GC-FID) retention times divided by 3 minus 3× GC-AED retention times for 36-compound subset of the library.

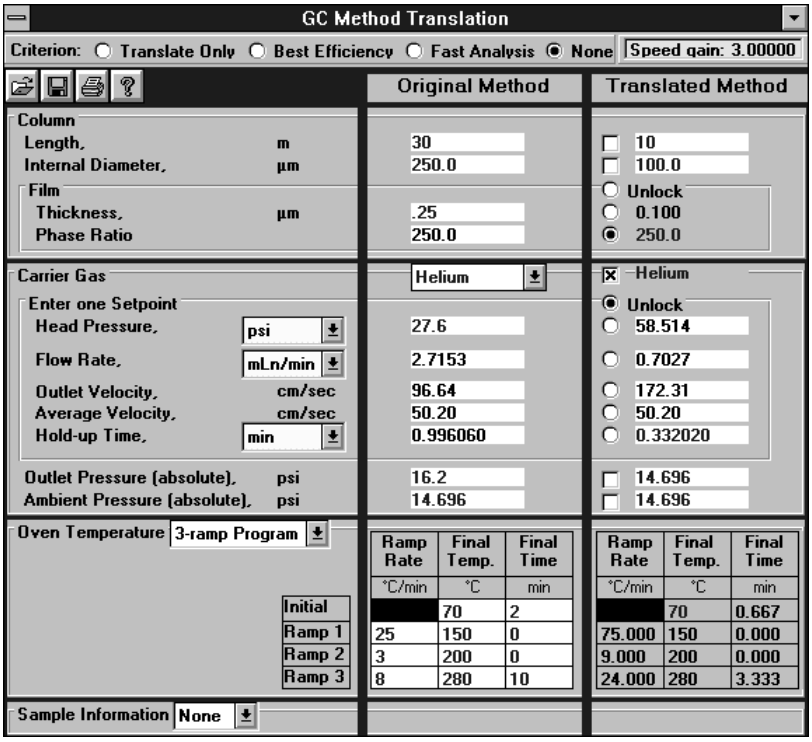

Figure 7. Method translation software showing scaling RTL pesticide method scaled to a threefold faster method on a 10-m × 0.1-mm id column.

Table 7 shows a comparison of locking pressures calculated using measured and predicted 3× 0.1-mm id micro-ECD calibration data. The range of locking pressures from the measured data (66.03 to 65.93) only corresponds to a spread in retention times of about 0.004 minute. However, with the data calculated based on a 10-m assumed length, the spread (66.38 to 63.18) is much larger and would correspond to a time range of 0.14 minute. The locking pressures calculated using the 10.5622 value are much more consistent with the measured values. The range in retention times would be ~ 0.03 minute if all the calculated points are used, and if the first value in column 5 is ignored, the range drops to ~ 0.005 minute.

The fact that the agreement in locking pressures is much improved by using 10.56 m instead of 10 m suggests that length is probably the largest contributor to the discrepancy. These results should reinforce the recommendation that if a method is to be used extensively, it is prudent to obtain measured RTL calibration data. It should be noted, however, that even with the RTL calibration from the 10-m assumed length, the worst consequence would be that the RT locking step would need to be repeated an extra time to get a more precise match.

Figure 8 compares the chromatograms of the RTL locking mixture from both the original and the 3 × 0.1-mm id micro-ECD methods.

Table 5. RTL Calibration Points from Original GC-AED Method and Calculated Points for 3× 0.1-mm id Micro-ECD Method Assuming 10-m Column Length

GC-AED RTL Calibration		3x Micro-ECD RTL Calibration	
Pressure (psi)	Ret Time (min)	Calculated Pressure (psi)	Calculated Ret Time (min)
33.1	15.346	71.03	5.115
30.4	15.919	64.90	5.306
27.6	16.578	58.51	5.526
24.8	17.338	52.11	5.779
22.1	18.242	45.91	6.081

Table 6. RTL Calibration Points from Original GC-AED Method and Calculated Points for 3× 0.1-mm id Micro-ECD Method Assuming 10.5622-m Column Length

GC-AED RTL Calibration		3x Micro-ECD RTL Calibration	
Pressure (psi)	Ret Time (min)	Calculated Pressure (psi)	Calculated Ret Time (min)
33.1	15.346	80.03	5.115
30.4	15.919	73.13	5.306
27.6	16.578	65.95	5.526
24.8	17.338	58.74	5.779
22.1	18.242	51.75	6.081

Table 7. Comparison of Locking Pressures Calculated Using Measured and Predicted 3× 0.1-mm id Micro-ECD Calibration Data

3x Micro-ECD Locking Runs		Locking Pressures		
Measured 3x Micro-ECD RTL Cal Points		Using Measured RTL Cal Points	Using 10-m Calculated RTL Cal Points	Using 10.56-m Calculated RTL Cal Points
Pressure (psi)	Ret Time (min)	Pressure (psi)	Pressure (psi)	Pressure (psi)
48.81	6.323	65.95	66.38	65.30
52.66	6.041	66.03	65.77	65.85
58.51	5.797	65.95	65.12	65.96
64.36	5.585	65.93	64.36	65.95
70.22	5.396	66.00	63.18	65.90

Note that while the most of the chromatographic resolution is preserved, the speed is increased by a factor of 3.

After being locked, the three peaks in the 3× micro-ECD method had retention times of 1.924, 5.533, and 9.963 minutes, respectively. These values are very close to the RTL Pesticide Library retention times for the three compounds divided by 3: 1.932, 5.532, and 9.949. The fact that the largest difference between the scaled table and the 3× micro-ECD method is only 0.014 minute again demonstrates the precision of retention time matching achievable with the scaling technique described here.

Conclusions

Using method translation combined with retention time locking provides a means of extending the usefulness of existing capillary GC methods. The ability to precisely scale a method to meet the needs of different samples and instrument types greatly reduces the effort required to re-use methods, thus saving time and money.

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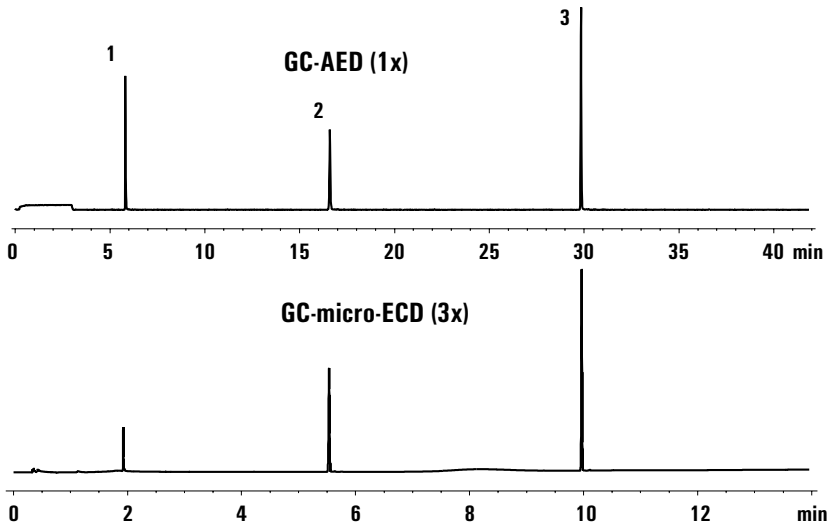

Figure 8. Chlorine chromatogram from 1× GC-AED method (top) and 3× micro-ECD method (bottom) of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.

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