

Retention Time Locking Using USP 467 Standard Sample and Automated Headspace Sampling

Application

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Abstract

Agilent Technologies' retention time locking (RTL) software gives chromatographers the ability to match retention times precisely from any Agilent 6890 Series or Agilent 6850 gas chromatograph (GC) system to any other, as long as the systems are equipped with electronic pneumatics control. Results from different systems are virtually identical, independent of inlet, detector, operator, or location. This application note explains the successful implementation of RTL for an automated headspace GC system.

Key Words

Retention time locking, RTL, USP 467, residual solvents, headspace, gas chromatography, 6890 Plus, Agilent 7694A

Introduction

Retention time is the primary tool for identifying peaks in gas chromatography. Peak identification is usually done by comparing the retention time of an unknown peak to peaks in a standard sample. It is critical, therefore, to minimize variation in the retention times of peaks to ensure proper peak identification and method validation.

Variation in retention time is common with gas chromatographs, even within a single GC system. Routine column trimming or a difference in column length from the nominal length changes peak retention time. For systems running duplicate methods, the resulting differences in retention times make it difficult to compare results. In these cases, reviewing or comparing data among systems is complicated and a new calibration table, integration events table, and timed events table may be needed.

Retention time locking enables chromatographers using the same nominal column to match retention times within thousandths of a minute in any 6890 Series GC or 6850 GC system equipped with electronic pneumatics control (EPC). EPC offers unwavering pressure and flow control. When combined with other factors such as stable and reproducible oven temperatures and highly consistent capillary columns, RTL becomes a very valuable tool for GC analysis.

With precise results from system to system, RTL can also be used as a system suitability assessment tool. If, for example, should system locking not be achieved for polar compounds, even though other compounds do lock, it becomes clear that there is some added system activity (such as inlet or column contamination).

It is common today to keep response calibration records and to update the integration timetable periodically for each column and each GC system. This is tedious and time-consuming. RTL presents a new option to match



all retention times from day to day and instrument to instrument in a manner analogous to re-zeroing a balance or re-calibrating a pH meter to the correct value.

Retention time locking is achieved by adjusting the column head pressure according to an RTL calibration table specific for each method. The USP 467 protocol suggests that carrier gas linear velocity should be "about" 35 cm/s. Therefore, the typical USP 467 SOP specifies a range for the inlet pressure (for example, 4.7 ± 0.5 psi) to accommodate inter-system and inter-column differences. The SOP can easily go one step further by specifying the retention time of a compound to simplify the calibration task and minimize timetable changes. For instance, ASTM method D 5134-92 requires the operator to "set carrier gas flow rate such that the retention time of toluene at 35 °C is 29.6 ± 0.2 min." If the target peak can be locked within the inlet pressure range, the system meets the system suitability criterion and is ready for analysis.

The RTL Process

The RTL calibration process involves correlating inlet pressure with the retention time of any selected target compound— the locking compound^{1,2}. This target compound, usually found in the normal method calibration standard, is then used to lock other GC systems. Agilent's RTL software (G2080AA) integrates into the Agilent ChemStation (version A.05.02 or later), providing the tool required to determine the proper inlet pressure for locking the retention time quickly and easily. The locking compound should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Polar and labile compounds should be avoided as locking compounds.

Once the target compound is chosen, the next step is to perform five calibration runs with different inlet pressures. The runs are made at identical conditions except for inlet pressure



Figure 1. Five calibration runs to generate the five pairs of inlet pressures and corresponding retention times of the target compound.

(as shown in figure 1). The pressures used are typically:

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

After the five runs are complete, the retention times and corresponding inlet pressures of the target compound from all five runs are entered into the ChemStation RTL software (G2080AA) to generate an RTL calibration file. Figure 2 shows the dialog box used to enter the calibration data. After the data are entered, the software program reveals the maximum retention time deviation determined from the fitted data curve, as shown in figure 3.



Figure 2. Dialog box used for entering retention time locking calibration data.



Figure 3. Plot of calibration data displayed by the RTL software.

The four calibration lines in figure 3 are as follows:

- 1. A point-to-point fit
- 2. The best curve fit of the five points
- 3. + 1 standard deviation from the best curve fit
- 4. 1 standard deviation from the best curve fit

If the fit is acceptable, the retention time versus pressure calibration is stored and becomes part of the GC method. This calibration needs to be generated only once, is stored within the method, and can then be used on a duplicate instrument, regardless of location.

Relocking a System or Locking Another GC

With the calibration stored in the GC method, the procedure for relocking a system or locking another 6890 or 6850 GC is easy:

- 1. Set up the method parameters, with nominal head pressure (for example 4.72 psi) and run a standard containing the target compound.
- 2. Enter the actual retention time of the target compound into the "(Re)Lock GC" dialog box (see figure 4).
- Press the "Calc. new pressure:" button to obtain the proper inlet pressure for locking.
- 4. Update the GC method with the new calculated pressure, and save the method.
- 5. Run the standard again at the new pressure, and compare the retention time obtained to the desired retention time.
- 6. Repeat steps 2 to 5, if necessary.



Figure 4. Dialog box used to calculate locking pressure and update the 6890 GC method (first lock). The numbers 2, 3, and 4 refer to the steps on the left.



Figure 5. The flow configuration for this study.

Experimental

A 6890 Plus GC with a 7694A headspace autosampler was used to verify retention time precision and reproducibility with RTL. The headspace autosampler transfer line was connected to the split/splitless (S/SL) inlet carrier line using a zero dead volume (ZDV) union (see figure 5). The standard sample was a USP 467 calibration mixture (USPM-4670, Ultra Scientific, North Kingston, RI) diluted 500:1 with water. The components and the final concentrations were: methylene chloride (10 ppm), chloroform (1 ppm), benzene (2 ppm), trichloroethylene (2 ppm), and 1,4-dioxane (2 ppm).

Results and Discussion

The target retention time for the locking compound was 4.296 min, as seen in figure 1. The inlet pressure was 4.72 psi (established now as the nominal method and pressure). Relocking the target peak was required after cutting off one meter of column (a common procedure). After cutting, the new retention time at 4.72 psi was 4.064 min. The 4.064 value was entered into the RTL software (see figure 4), which then calculated the new pressure to be 4.39 psi. At 4.39 psi, the retention time of the target peak improved to 4.314 min (versus the target 4.296 min). In this example, a second lock was needed to match retention times to within 0.001 min. Figure 6 shows the process of entering the current retention time (4.314 min) to get the desired new pressure (4.42 psi). Indeed, by using the inlet pressure of 4.42 psi, the retention time of the target peak was locked at 4.297 min, which was within 0.001 min of the target retention time of 4.296 min.

Inlet	Split/splitless (5:1 split), 200 °C, constant pressure, helium carrier gas
Column	HP-INNOWax polyethylene glycol, part no. 19095Ν-123, 30 m x 530 μm x 1 μm
Oven	50 °C (3.5 min), 10 °C/min to 60 °C (0 min), 40 °C/min to 190 °C (1 min)
Detector	FID, 300 °C, H ₂ = 40 mL/min, air = 450 mL/min, nitrogen makeup = 45 mL/min
Sample	5 mL in 10-mL headspace vial
Headspace zone temperatures	Oven = 85 °C, loop = 95 °C, transfer line = 100 °C
Headspace	Vial equilibration time = 15.0 min, pressure time = 0.30,
event times	loop fill = 0.15, loop equilibration time = 0.02, Injection = 0.50
Headspace vial pressure	15 psi
ChemStation	Version A.06.01, RTL software G2080AA



Figure 6. Dialog box used to calculate locking pressure and update the 6890 GC method (second lock). The numbers 2, 3, and 4 refer to the steps on page 4.

Figure 7 overlays the four GC runs discussed above.

Retention time locking is applicable even for regulated environments, for cases when columns are not changed or trimmed for extended periods of time, or when results are not compared from system to system. Besides providing the capability to match retention times within thousandths of a minute, RTL helps confirm system suitability quickly and easily:

- 1. *When locking a new system*, if the locking for some or all of the peaks gives a poor fit (a large delta RT), the system is not working properly.
- 2. *When using a locked method*, any shift in retention time (caused by leaks or flow restrictions) signals a system problem.
- 3. *After changing or trimming a column*, if relocking cannot be achieved after two attempts, the system should be carefully examined for problems.

The SOP can specify that the retention time of a reference peak should be xx.xx minutes ± 0.2 minute, for example. The ability of a system to maintain a fixed retention time for the reference peak provides high confidence in peak identifications and affirms good system performance. Moreover, when the retention time is locked, anomalies with peak shape, peak width and peak resolution are recognized more easily as immediate signs of system problems.



Figure 7. Comparison with the original chromatogram after one meter of column was cut off, and after two RTL operations.

Conclusions

In chromatography, most peak identification is performed by comparing the retention time of an unknown to that of a standard. It is much easier to identify peaks and validate methods if there is no variation in the retention time of each compound after column installation or trimming. Retention time locking can also help to ensure system suitability before samples are run.

This study demonstrates that RTL can be applied to a headspace-GC system.

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