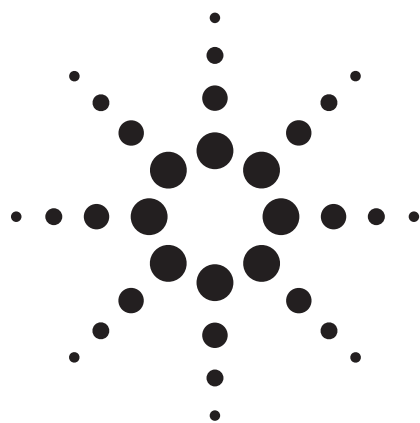# Retention Time Locking with the MSD Productivity ChemStation

Technical Overview

Introduction

A retention time is the fundamental qualitative measurement of chromatography. Most peak identification is performed by comparing the retention time of the unknown peak 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 analyte.

However, shifts in retention time occur frequently. Routine maintenance procedures such as column trimming alter retention times. In a multi-instrument laboratory running duplicate methods, the retention times for each instrument will differ from each other, even when run under nominally identical conditions. These differences in retention times mean that each instrument must have a separate calibration and integration event table, making it time-consuming to transfer methods from one instrument to another. Differences in retention time also complicate comparison of data between instruments over time.

What Is Retention Time Locking?

Retention time locking (RTL) allows a close match of retention times on one Agilent Technologies GC/MSD or GC system to those on another like system with the same nominal column. By making an adjustment to the inlet pressure, the retention times on one system can be closely matched to those on another system using the same nominal column. The ability to very closely match retention times from one system to another can greatly reduce the time it takes to develop and transfer methods. "RTLocked" methods can also compensate for degradations in chromatographic perfor-

mance. The ability to correct for degrading chromatographic performance, optimize lab resources, and still provide the correct answer saves time, money, and results in significant productivity gains.

Using GC/MS, it is also possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The RTL mass spectral database provides additional confirmatory information in spite of changes to the chromatographic system. One such database is the G1672AA Pesticide RTL Library. This database allows for quick and easy screening of pesticide samples.

When Should I Lock My Methods?

Locking or relocking your methods should be done whenever you make any changes to the chromatographic system or move methods from one system to another (GC or GC/MS). To establish and maintain a locked method, RTL should be performed whenever:

- The column is changed or trimmed
- The method is installed on a new instrument
- A detector of different outlet pressure is used (GC vs. MS)
- System performance is validated
- Troubleshooting chromatographic problems

How Does It Work?

The process of RTL is to determine what adjustment in inlet pressure is necessary to achieve the

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desired match in retention times. To lock a given method for the first time or for the reasons above, you must first develop a Retention Time vs. Pressure (RT vs. P) calibration curve. Using an established method, multiple (five) injections of the standard are used to calculate the retention times at predefined inlet pressures. The use of an automatic sampler simplifies this process. The RT vs. P calibration data for each of the five injections are saved and used to correct locked methods.

The five defined pressures are:

• Target pressure –20%

• Target pressure –10%

• Target pressure (nominal method pressure)

• Target pressure +10%

• Target pressure +20%

Even when using columns with the same part number (same id, stationary phase type, phase ratio, and same nominal length), separate and different locking calibration curves may be needed. Other examples of when separate locking calibration curves are required include:

• Systems with different column outlet pressures (MSD/vacuum, FID/atmospheric)

• Columns differing from the "nominal" length by more than 15% (for example, due to trimming)

• Systems where the predicted locking pressure falls outside the range of the current calibration

Selecting the Standard Compound for RTLocking

A specific compound (usually one found in the normal method calibration standard) must be

chosen and then used for both developing the locking calibration and locking all future systems. The compound, or target peak, should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Compounds that are very polar or subject to degradation should be avoided.

Once the target compound has been chosen and all other chromatographic parameters of the method have been determined, the five calibration runs are performed. The resulting RT vs. P calibration curve data are saved. The software is then used to select and integrate the peak used for locking.

Creating an RTL Method

The following is an overview of the actual steps taken to acquire the RT vs. P calibration curve data, selecting the compound/peak to use and locking the method.

Acquiring the RTLock Data

From **Instrument Control**, select the **Instrument** menu and

Acquire RTLock Calibration Data.... Agilent Technologies GCs are the only ones supported. See Figure 1.

If you are using an automatic sampler, the five sample injections will be made automatically. This example illustrates how RTL works on a GC/MS system. Upon completion of the five-sample analysis, Data Analysis will begin and display the nominal MS total ion chromatogram (TIC). If the system was configured as a stand-alone GC, all five chromatograms will be displayed. From these displays, you will select the compound that will be used for RTLocking of the method.

Selecting the RTLock Compound

Use the mouse to select the compound or peak that you would like used for locking. For GC-only mode, you must select one peak from each of the five chromatograms for RTLocking. Once you have made your selection, you will be asked to allow the software to automatically find the remaining peaks. You may choose to zoom the display for better visibility. See Figure 2.
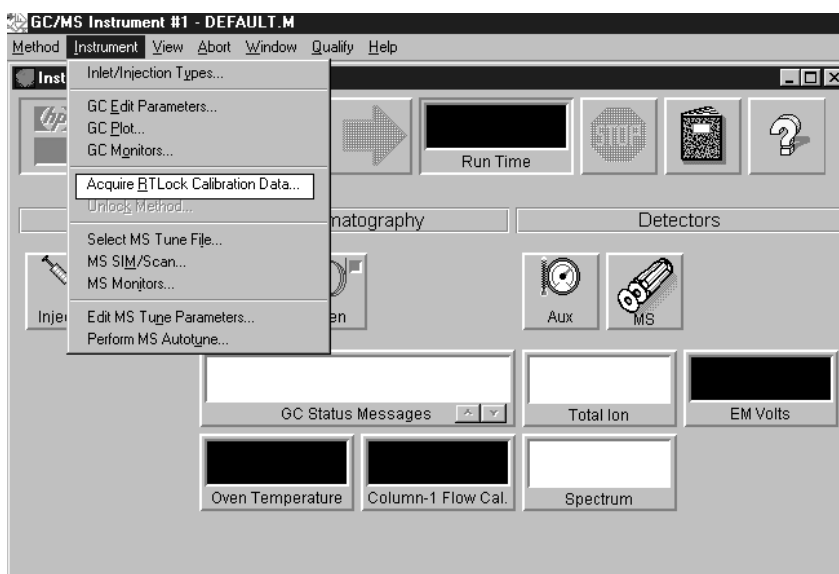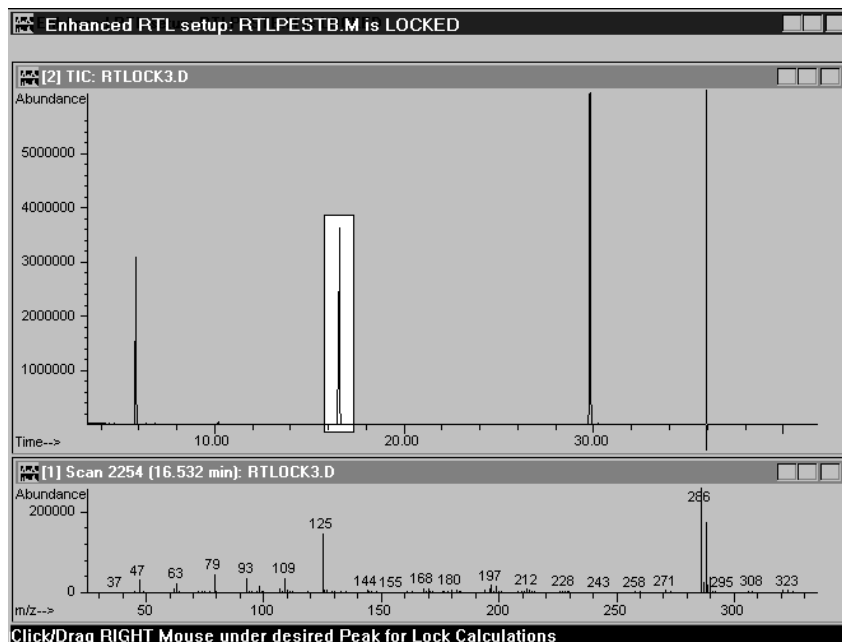

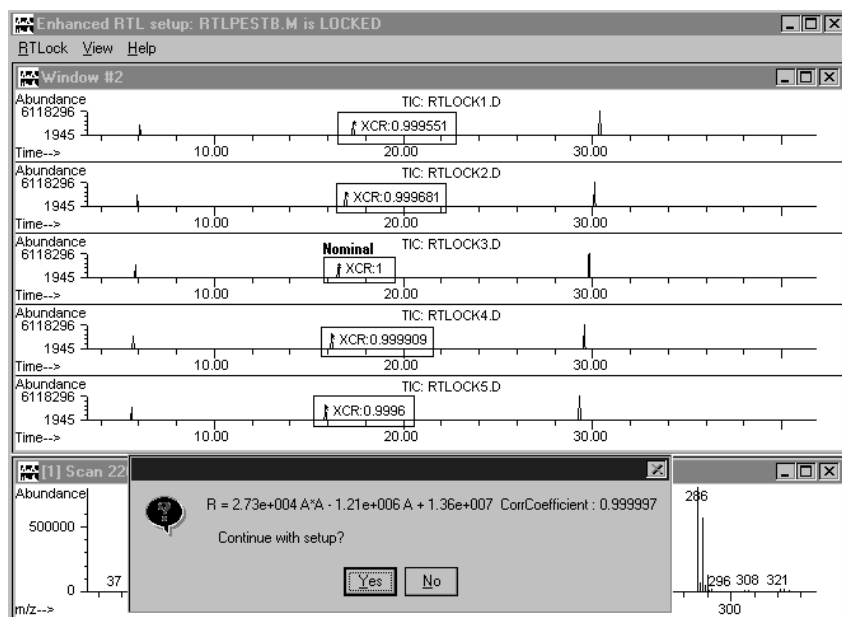Figure 1.   From instrument control, you select the mode of data acquisition for RTL.

Figure 2. From this panel, the user selects the peak used for RTL. In this example the second peak has been selected. The spectrum of the selected compound is also displayed and is used to confirm the RTL compound.
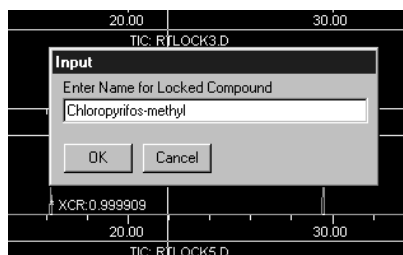

Figure 3. When the RT vs. P calibration curve equation is calculated, the correlation coefficient is determined for the RTLocked compound in each of the five calibration samples. The resulting coefficient is displayed at each peak. The "nominal," or no change to pressure calibration sample, has a correlation of 1.

Calculating the RTLock Curve and Saving a Method

Once the RTLock compound has been selected, the new RT vs. P curve for each compound will be displayed. To select a new compound and calculate new RT vs. P curves, reset the nominal MSTIC from the **RTLock** menu item. Select a new compound as the RTL compound and from the **RTLock** menu item, select **Calculate New Curve from Selected Peaks**. This will generate new RT vs. P calibration curves. The new RT vs. P calibration curve equation will be displayed on the screen along with the correlation coefficient. Select **Yes** to either create a new, or update an existing, RT vs. P calibration file. See Figure 3.
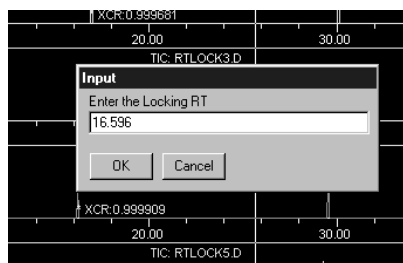
Next, you can enter the name and retention time of the RTLock compound. See Figure 4 and Figure 5.
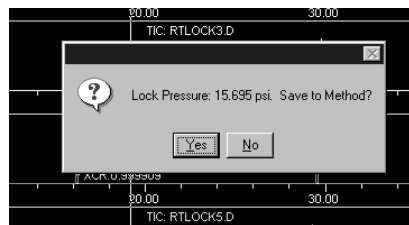

Figure 4. Enter or confirm the name of the RTLock compound.

Figure 5. Enter or confirm the retention time of the RTLock compound.

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You will then be asked to confirm the RTLock pressure that has been calculated and will be used for that method. Select **Yes** to confirm the new RTLock pressure and save the method. See Figure 6.

Figure 6.   By selecting Yes, you RTLock and save the method.

View Current RTLock Method Setpoints and Report

Once the system is calibrated and locked, you can view and confirm the RTLock setpoints by selecting **View Current Method Setpoints.** See Figure 7.
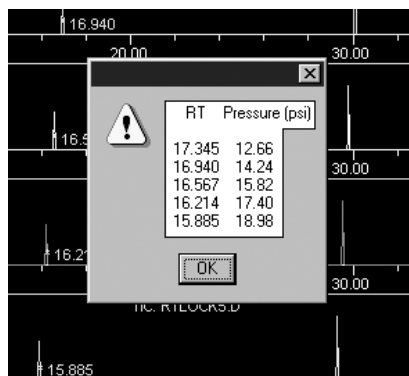
Figure 7.   Viewing the RTLock retention times and corresponding pressures for the RTLock method.

A report is also available that provides detailed information regarding the RTLock method. See Figure 8. The report includes:

- Method name
- Calibration date
- Instrument name
- Operator name
- Status of method (on or off)
- RTLock compound name
- Tabular retention time/ pressure calibration table
- Maximum deviation
- RTLock curve equation and correlation coefficient data
- Locked retention time information (file name, acquisition date, instrument name, and operator name)
- Report date
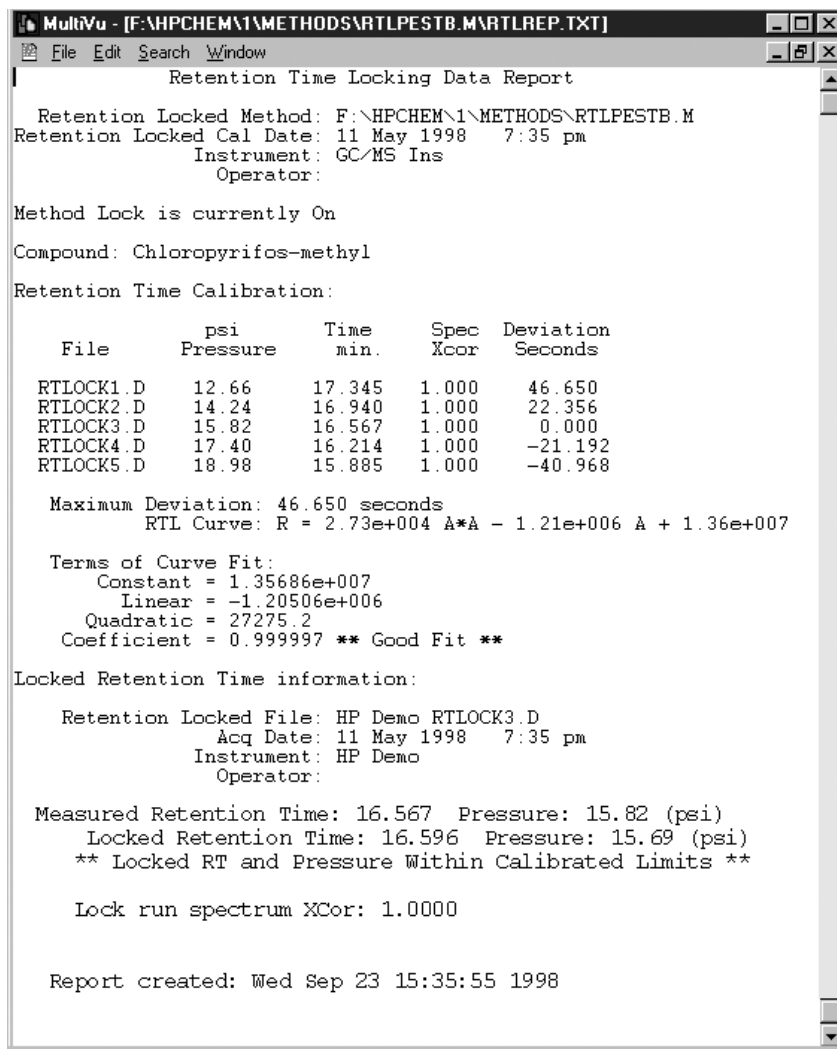
Figure 8.   Example RTLock Report

Running an RTLock Method

Once the RTL method is created, you can analyze and process new samples. This requires that you unlock and relock the methods without editing the quantitation calibration data. The following example demonstrates how retention times might change when column maintenance is performed or a method is moved to a new GC/MS system. See Figure 9. Once the method is RTLocked, new samples are analyzed and retention times corrected. See Figure 10.

Unlocking and Relocking a Method

Once a method is locked you may unlock or relock it using the same or different compounds or after additional maintenance. To define a new compound for RTLocking, select **RTLock Setup** from the **View** menu in **Data Analysis**. See Figure 11. The nominal RTLock sample that represents the method you are working on will be displayed. Once again, use the mouse to identify a new RTLock compound.
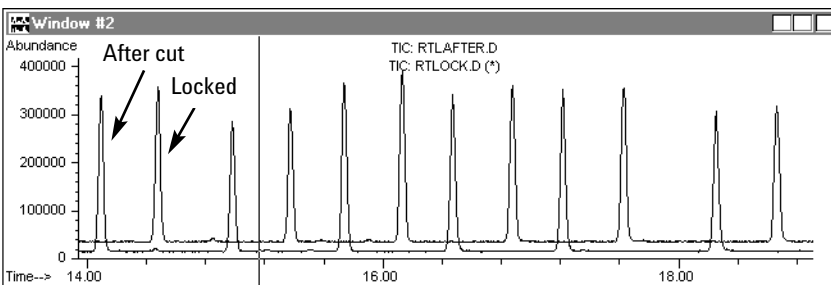

Figure 9. Overlay of an RTLocked data file (labeled Locked) and the resulting data file after clipping one meter off the column (labeled After Cut).
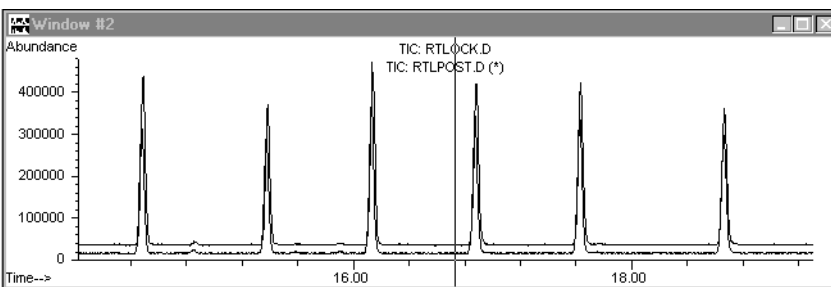

Figure 10. Offset overlay of RTLocked data file and the RTCorrected datafile show peaks overlapped and corrected for column maintainence.
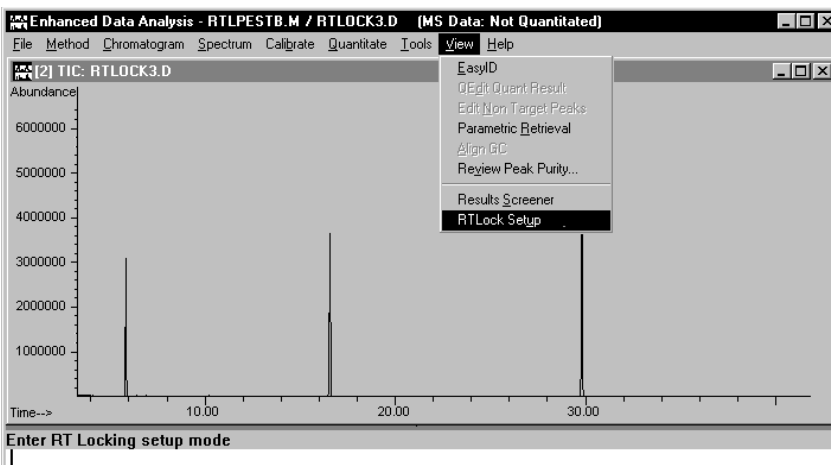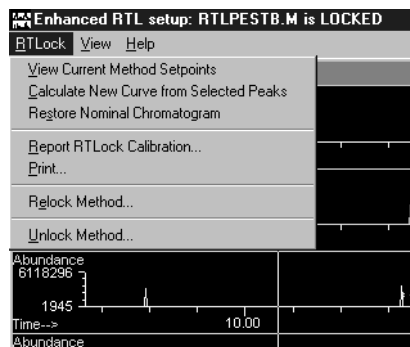

Figure 11. To define a new compound for RTLocking, select RTLock Setup from the View menu in data analysis.

After selecting the compound to RTLock, from the **RTLock** menu item, select **Calculate New Curve** from **Selected Peaks**. This will generate a new RT vs. P calibration curve. The new RT vs. P calibration curve equation will be displayed on the screen along with the correlation coefficient. Select **Yes** to create a new or update an existing RTLock calibration. See Figure 12. From the RTLock menu you can also:

• View current setpoints

• Calculate the RT vs. P curve

• Restore the original chromatograms

• Report the RTLock calibration

• Unlock the method

• Relock the method

Summary

RTLocking provides an easy and flexible tool that can be used to reduce the time and complexity often associated with routine chromatographic maintenance. It allows methods to be transferred between like GC/MS systems without time-intensive edits to the quant database and reacquisition of standards. It also simplifies the process for executing routine chromatographic maintenance. RTLocking can minimize mistakes and provide a productivity improvement for most applications by reducing the time and setpoint changes required to update a method.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Figure 12.   RTLock view and menu choices.

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