Enhanced Reliability of Forensic Drug Testing Using Retention Time Locking

Application

Gas Chromatography

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Abstract

Retention time locking (RTL) is used to lock retention times when transferring methods from chromatographic instrument to chromatographic instrument, column to column, and detector to detector. The retention times for a derivatized cocaine standard were locked when the method was translated from an Agilent 6890/Agilent 5972 GC-MSD (gas chromatograph-mass selective detector) system to other Agilent GC-MSD systems and when performing column trimming as a routine maintenance procedure on the same system. The retention times, under both circumstances, matched the original retention times within 0.01 minute after locking.

Key Words

Retention time locking, RTL, method validation, forensic drug testing, capillary gas chromatography, MSD, laboratory productivity, cocaine.

Introduction

In large drug testing laboratories, several instruments are used simultaneously to analyze or screen for cocaine and/or its derivatives. To increase laboratory productivity and improve ease of sample identification and quantitation, laboratories could benefit in many ways by matching retention times of analytes and internal standards from instrument to instrument, day to day, and location to location. This is easily achieved using the G2080AA retention time locking (RTL) software tool for GC systems with electronic pneumatics control (EPC) using the Agilent GC ChemStation (version A.05.02).

RTL provides the ability to match chromatographic retention times exactly in any 6890 GC system to those in another chromatographic system with the same nominal column. The RTL software allows rapid, accurate locking of all retention times using columns of the same stationary phase and dimensions (same part number). The concepts, uses, and requirements for RTL have been discussed elsewhere.[1] This application note examines the use of RTL to prepare the retention time vs. pressure (RT vs. P) calculation for a derivatized cocaine standard sample from a method that was optimized for a 6890/ 5972 GC-MSD system. Retention times for the derivatized cocaine sample were easily locked when the same method was transferred to a 6890/Agilent 5973 GC-MSD system or an 5890/5972 GC-MSD system. In addition, retention times of target cocaine derivatives were again locked every time when column trimming was performed in the same GC system.

At the time of this study, the software used to accomplish locking was not available for the GC/ MSD system. A duplicate method was created on a separate GC ChemStation to develop the RTL relationships for the data collected from the GC/MSD systems. The RTL software residing on the GC ChemStation was also used to calculate the recommended locking pressure for the GC/MSD system based on the RT vs. P relationship and the results from the GC/MSD scouting run.

Experimental

Samples of benzoylecgonine (BE) containing cocaine and cocaine-d3 (internal standard) were prepared and analyzed in accordance with methods reported previously.[2,3]

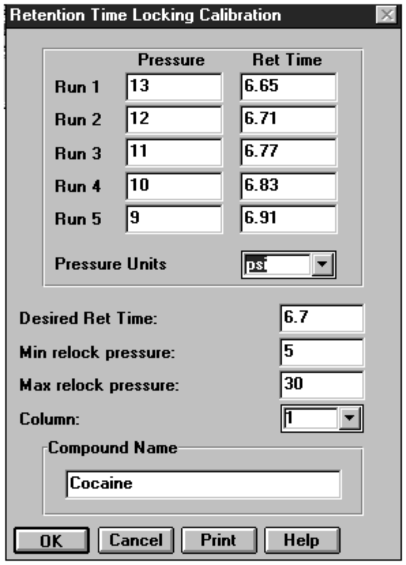
For the analysis of trimethylsilyl derivatives of BE samples, GC systems equipped with split/splitless inlets (splitless mode) and mass selective detectors (5972 MSD or 5973 MSD) were used. A GC/MSD ChemStation was used for instrument control and data acquisition. The experimental conditions for the GC methods are given in table 1.

Table 1. Experimental Conditions

Automatic sampler	Agilent 7673B, 10-µL syringe, 1-µL injection, viscosity delay: 1 sec
Inlet	Split/splitless inlet, 250 °C, splitless mode
Carrier	Helium, constant pressure (1.2 mL/min at 150 °C), vacuum compensation: on
Column	Agilent HP-5MS, 30 m x 0.25 mm x 0.25 µm (part no. 19091S-433)
Oven	150 °C (0.5 min), 20 °C/min to 290 °C (4 min)
Detector	Direct column interface to MSD at 290 °C, autotune with 600 V above autotune voltage, SIM mode with 25-second dwell time
Software	G2070AA GC ChemStation A.05.02, G2080AA RTL software for GC ChemStation, and G1701AA MSD ChemStation A.03.00

Results and Discussion

In this study, a testing laboratory wanted to lock the retention times of the trimethylsilyl derivative of cocaine and its internal standard at 6.70 minutes on any Agilent GC-MSD system. The chemist developing the locking method first ran five analyses using different column head pressures to establish an RT vs. P relationship. The RT vs. P data were manually entered into the RTL software resident on a separate GC ChemStation. The RTL software RT vs. P calibration screen is shown in figure 1. The RT vs. P information is saved in the method and is valid for any Agilent GC system using the same method and nominal column. The result of this RT vs. P relationship is shown in figure 2. Once this relationship is developed, it can be used to lock any other system of the same configuration. To do that, the method is loaded and a scouting run is done at the method's nominal pressure.



	Pressure	Ret Time
Run 1	13	6.65
Run 2	12	6.71
Run 3	11	6.77
Run 4	10	6.83
Run 5	9	6.91

Pressure Units: psi

Desired Ret Time: 6.7

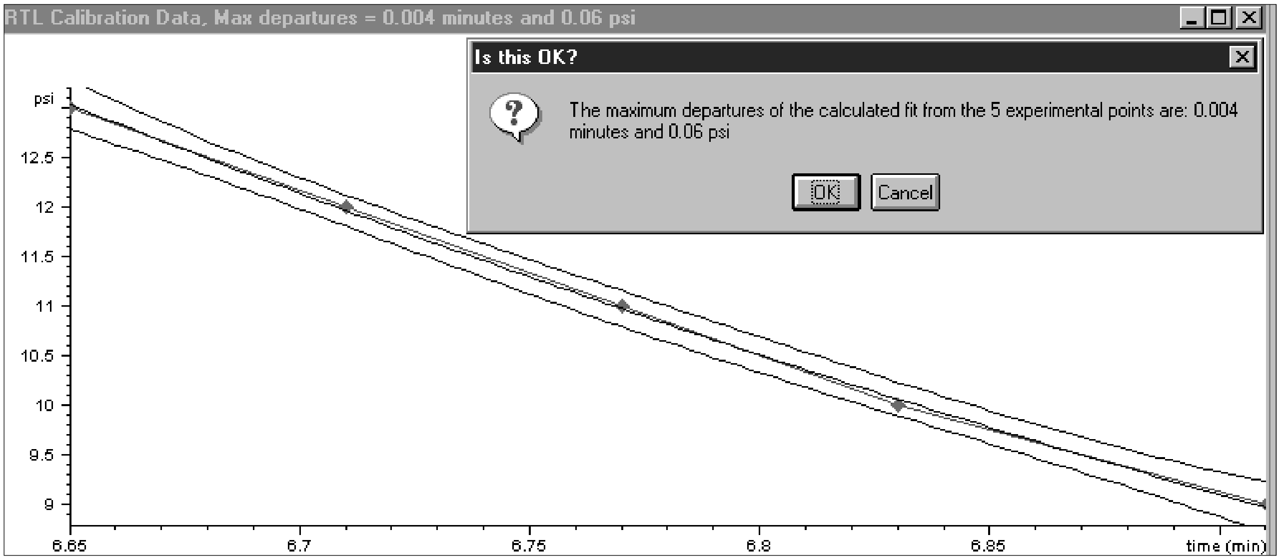
Min relock pressure: 5

Max relock pressure: 30

Column: 1

Compound Name: Cocaine

Figure 1. Preparation of retention time locking calibration using the Agilent GC ChemStation.


RTL Calibration Data, Max departures = 0.004 minutes and 0.06 psi

Is this OK?

The maximum departures of the calculated fit from the 5 experimental points are: 0.004 minutes and 0.06 psi

Figure 2. RT vs. P relationship for retention time locking.

On system I (6890/5972 GC-MSD), a scouting run using a head pressure of 12.00 psi yielded a retention time of cocaine at 6.71 min (see table 2). Based on this data set, the RTL software (on the separate GC ChemStation) recommended a column pressure of 12.17 psi for the method (see figure 3).

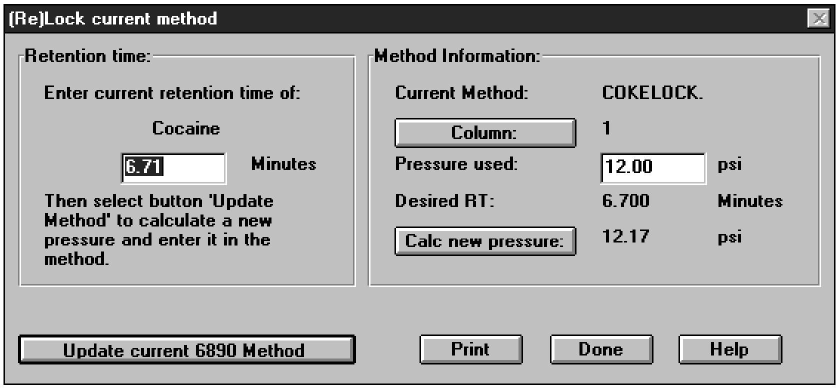
A column pressure of 12.2 psi was manually entered into the GC/MSD and the extracted ion chromatograms shown in figure 4 were produced. The RT for cocaine was found at 6.69 minutes. In the next five runs at 12.20 psi, the retention time for cocaine was reproducibly obtained at exactly 6.69 min. Extracted ion chromatograms of these runs (m/z 240/256 and 243/259) confirmed that the peak eluting at 6.69 min was indeed cocaine and its internal standard, cocaine-d3 (see figure 4).

Similarly, a good RT match was obtained on system II (6890/ 5973 GC-MSD system) shown in table 2. The same technique was used to

Table 2. Retention Time Locking When Transferring a Method to Different Systems

	Scouting Run		(Re)Lock Run	
	Pressure (psi)	Retention Time(min)	Pressure (psi)	Retention Time(min)
System I	12.00	6.71	12.20	6.69
System II	12.20	7.57	9.10	6.70
System III	12.20	7.33	18.77	6.80
			20.39*	6.69*

* Second (Re)Lock run to fine-tune the method because the experimental retention time falls outside the RT vs. P curve



(Re)Lock current method

Retention time:

Enter current retention time of:

Cocaine

6.71 Minutes

Then select button 'Update Method' to calculate a new pressure and enter it in the method.

Method Information:

Current Method: COKELOCK.

Column: 1

Pressure used: 12.00 psi

Desired RT: 6.700 Minutes

Calc new pressure: 12.17 psi

Figure 3. Column pressure recommendation for retention time locking and method update (as found on the RTL software for the GC ChemStation).

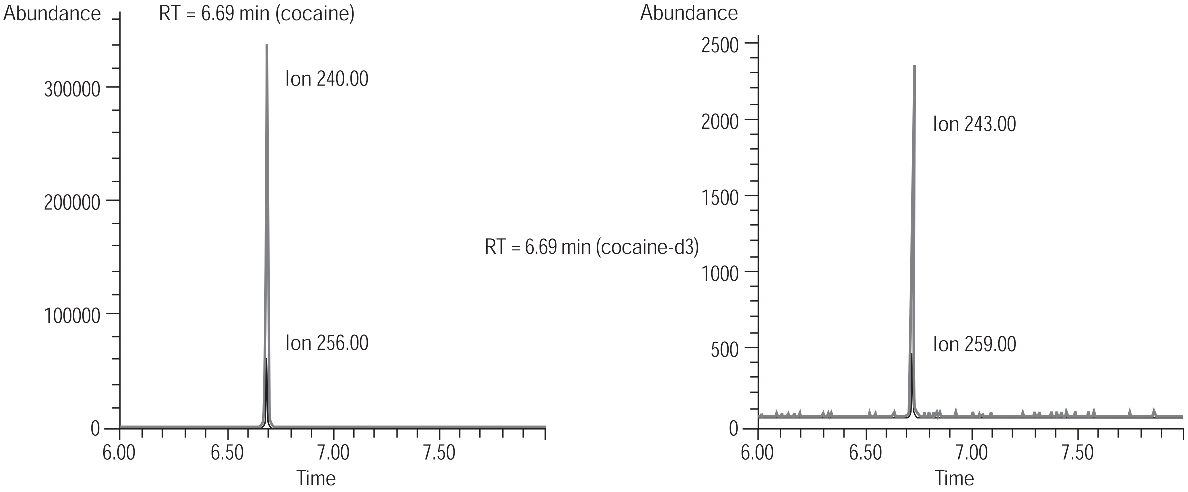


Figure 4. Extracted ion chromatograms of derivatized cocaine and internal standard (cocaine-d3) on System I (6890/5972 GC-MSD)

match RT on system III (5890 GC/5972 MSD system)-also shown in table 2. The results shown suggest that system III may have experienced some hardware irregularity. However, the RTL software was able to correct for minor system trouble and to provide recommendations for matching RTs after a third trial to finetune RT matching (see table 2). The recommended column head pressure of 20.39 psi was used to obtain a match for cocaine at 6.69 min for system III. The extracted ion chromatograms confirmed that the analytes that eluted at 6.69 minutes at this column head pressure of 20.39 psi on system III were cocaine and its internal standard, cocaine-d3 (see figure 5).

A routine system maintenance task such as cutting a short length from the head of the analytical column is performed regularly in testing laboratories. The RTs of cocaine were closely matched after each column trimming was performed (see table 3). Each time, a scouting run followed by a (Re)Lock run were performed and an RT of 6.70 min was achieved for cocaine.

First, cocaine was locked at 6.69 min with a pressure of 12.20 psi on system I using an Agilent HP-5MS column with a nominal length of 30 meters. When 14 inches of the column were trimmed off, a scouting run at 12.20 psi yielded an RT of 6.66 min for cocaine. To lock the system, the RTL software suggested a new column pressure of 11.48 psi. The (Re)Lock run yielded an RT of 6.70 min for cocaine (see table 3), and the system was locked again.

The column was trimmed a second time by an additional 14 inches, and a scouting run followed by a (Re)Lock run were performed. The resulting RT for cocaine was locked at 6.70 min with the recommended pressure of 11.00 psi. Finally, the column was trimmed by an additional 28 inches (for a total of 56 inches cut from the 30-meter column). Again, the RT for cocaine was easily locked at 6.70 min with a column pressure of 9.10 psi, which was recommended by the RTL software and manually entered into the GC/MSD (see table 3).

Table 3. Pressures and Retention Times after Performing System Maintenance on System I

Tasks	Scouting Run		(Re)Lock Run	
	Pressure (psi)	Retention Time (min)	Pressure (psi)	Retention Time (min)
Column length, 30 meters			12.20	6.69
Cut 14 inches (30 m - 14 in.)	12.20	6.66	11.48	6.70
Cut additional 14 inches (30 m - 28 in.)	11.50	6.66	11.00	6.70
Cut additional 28 inches (30 m - 56 in.)	11.00	6.60	9.10	6.70

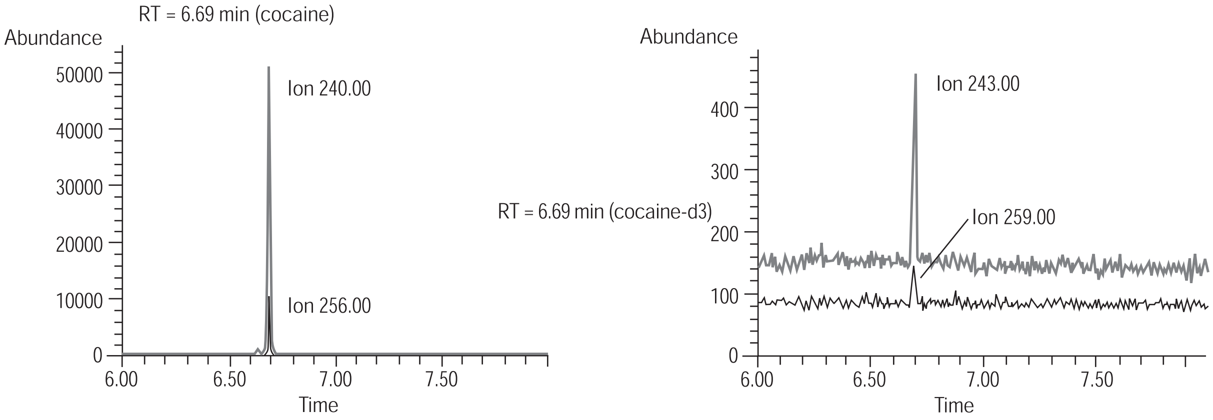


Figure 5. Extracted ion chromatograms of derivatized cocaine and internal standard (cocaine-d3) on System III (5890/5972 GC-MSD).

Conclusions

The G2080AA retention time locking software tool was used to lock retention times for a derivatized cocaine sample analyzed on three different GC/MSD systems and after column trimming. The retention times under these circumstances matched the original retention time within 0.01 minute. The ability to lock retention times means that one calibration can be used for multiple systems, and data from the past can be easily compared to new data.

References

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