The Importance of Area and Retention Time Precision in Gas Chromatography

Technical Note

Abstract

Area and retention time are the two primary measurements in gas chromatography. The precision with which they are measured ultimately determines the validity of the generated quantitative data. Key parameters affecting their precision are discussed.

Introduction

Two measurements govern all chromatographic data: retention time (t_R) and peak area. Precise t_R measurement is the mainstay of all modern gas chromatographs. Increasing t_R precision leads to increased confidence in qualitative reporting as well as an increased use of automation in quantitative analysis. The importance of t_R precision takes on greater significance as chromatographic efficiency increases (the use of small diameter capillary columns) and/or analysis time decreases (fast chromatography). Both of these conditions result in a potential for a large number of eluents per unit time. A lack of precision in t_R can easily lead to misidentifications, resulting in gross errors by matching the calibration data with the wrong analyte.

In gas chromatography (GC), t_R precision is controlled by both carrier flow and oven temperature precision. Secondary factors include auto-sampler speed and the precision of the “start run” signal.

The Agilent 6890N is capable of t_R precision better than 0.0008 minutes (0.048 seconds). With measurements this precise, small changes in ambient operating conditions (laboratory temperature and pressure) can dramatically affect retention precision. For this reason, the Agilent 6890N uses ambient pressure and temperature compensation.

Area precision ultimately determines the degree of accuracy of a quantitative measurement. While not directly related to t_R, area precision can be affected by many of the same variables affecting t_R. Precise flow control through a detector is critical for the maintenance of precise responses. The temperature of the detector also plays a part in determining some detector responses; the electron capture detector (ECD) or thermal conductivity detector (TCD) are more sensitive to temperature than others. Other factors influencing area repeatability are auto-sampler draw precision, speed of injection, and syringe performance. For active compounds, the inertness of the entire flow path can dramatically affect area precision, especially at low levels.

In short, precision of both t_R and area in GC are influenced by many parameters. Some of the parameters are more obvious than others. Retention time and area precision, compatable with high-efficiency and/or fast chromatography, require that all parameters (temperature, pressure, and flow) are controlled to exacting tolerances.

6890N Area and t_R Precision Specification

t_R Precision

The t_R precision of the Agilent 6890N GC is specified at better than 0.0008 minute or better than 0.008% RSD (relative standard deviation). The "or" between the two specifications is a true Boolean, meaning that one of the two conditions will always hold, and in most cases both will hold. These specifications were determined using the conditions listed in Table 1. In order to meet these specifications the instrument must be powered up, with flows, inlets, and detectors at temperature for at least 2 hours. The column is conditioned and is cleaned of any residual contaminants by performing at least one blank run (with no sample injection) immediately prior to test. No further equilibration of the instrument is necessary to meet these specifications. The resulting chromatogram is shown in Figure 1. Tetradecane (2 ng on-column was used for both the t_R and area precision specifications.

Table 1. Gas Chromatograph Conditions

Agilent 6890N GC with 7683N ALS				
5-µL syringe:	p/n 5181-1273			
Crimp-cap vials:	p/n 5181-1210 and crimp caps (p/n 5181-3375)			
Inlet EPC:	Split/Splitless			
Mode splitless:	1.0 µL injection			
Inlet temp:	250 °C			
Purge flow:	40.0 mL/min			
Purge time:	0.5 min			
Constant flow:	6.5 mL/min			
Gas saver:	Off			
Carrier gas:	Helium			
Detector FID				
Temperature:	300 °C			
H_2 flow:	30 mL/min			
Air flow:	400 mL/min			
Makeup flow:	25 mL/min			
Flame on offset:	<15 pA			
Makeup gas:	N_2			
Inlet Liner				
Agilent multi-purpose inlet liner	p/n 5183-4647			
Septum:	p/n 5183-4757			
Oven	120 V or 240 V			
	Oven ramp	**°C/min**	**Next °C**	**Hold min**
	Initial	–	75	0.5
	Ramp 1	20	190	0.00
Total run time	6.25 min			
Equilibration time	1.0 min			
Column				
Agilent HP-5.625, p/n 19091J-413, 30 m × 0.32 mm, 0.25 µm				
Agilent ChemStation	(32-bit)			
Signal 1 set to FID at a data rate of 5 Hz				
Integration Events Table				
Initial slope sensitivity:	5			
Initial peak width:	0.02			
Initial area reject:	5			
Initial height reject:	5			
Initial shoulders:	Drop			
Sequence				
At least one blank run				
Run sample six times				

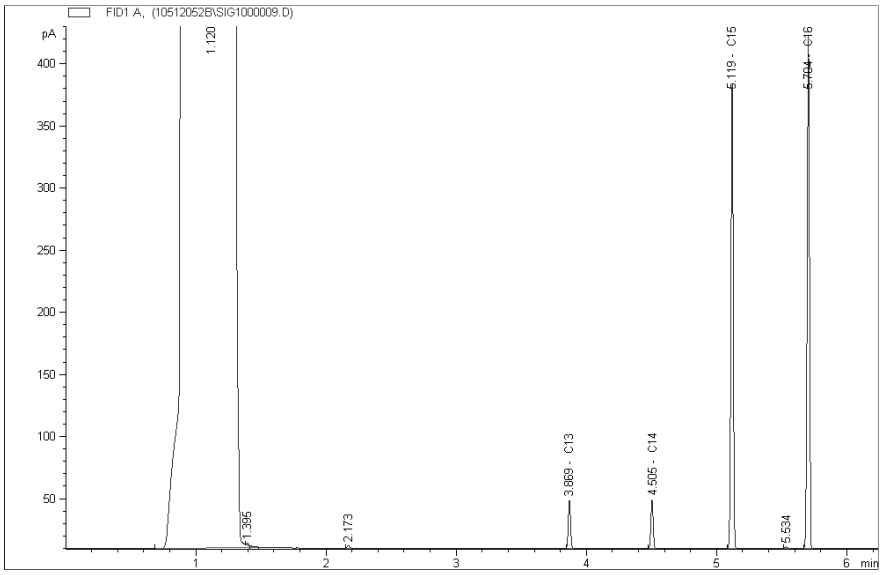

Figure 1. Test chromatogram.

The instrument conditions chosen are generic, meaning they are representative of many different analysis methods. Great care was also taken not to use conditions or procedures that would not be encountered in the normal day-to-day operation of a GC. Chromatography, like many measurement techniques, is highly dependent upon the conditions chosen by the operator. The conditions and results of the specifications are representative of what can be done by an average operator performing a typical method.

How Precise Do GC Measurements Need To Be?

As we move towards faster chromatography, peaks get narrower and the need for better t_R control increases. We know from theory that

$$t_r = t_m(1+k)$$

where:
t_r is the t_R
t_m is the t_R of an un-retained peak
k is the partition ratio
t_m is a function of the average linear velocity $\bar{\mu}$
L is the column length

$$t_m = \frac{L}{\bar{\imath}}$$

Now $\bar{\mu}$ is a function of the pressure drop across the column and the viscosity of the gas, which has a temperature dependence. So for a given column, $\bar{\mu}$ will change with changes in both temperature and pressure.

k is a thermodynamic function with dependence on the film thickness (capillary columns) and the temperature of the column. So to achieve good t_R repeatability, we must control column temperature and linear velocity. Changes in film thickness will also effect this, but these changes are usually slow, unidirectional (the film gets thinner), and are not an instrument parameter subject to our control other than an initial choice of film thickness in the column.

The need of accurate t_R precision is a function of peak width and resolution. The primary use of t_R is to identify peaks. If the t_R of two peaks move so much that they can't be correctly identified (fall outside a set t_R window) then there is too much variation. Consider for instance, the two peaks illustrated in Figure 2, with a resolution of 1 and equal peak widths:

From the formula for resolution, R, we know that

$$R = 1 = \frac{t_{r2} - t_{r1}}{w}$$

In this expression, w is the peak width at the base:

$$w = 4\sigma$$

where σ has the usual relationship for a Gaussian peak:

$$y = \frac{Ae^{-(t-t_r)^2/2\acute{o}^2}}{\acute{o}\sqrt{2\eth}}$$

It should be noted that the peak width at half height, $w_{1/2}$, which is usually measured, is given by:

$$w_{1/2} = 0.589w = 2.355\sigma$$

Now for our two peaks, $t_{r2} - t_{r1} = w$. To be able to confidently say that a peak is either 1 or 2, we must be confident that neither peak can move more than half the distance between them. Another way of saying this is that our t_R window for identification is less than ±w/2. Note that this is an absolute, not a relative window. It is not uncommon for peaks to exhibit $w_{1/2}$ of 60 ms with the use of a 100- or 150-µm column or larger diameter columns operated well above optimal flow in "fast" mode. Therefore, t_R repeatability must be better than ±60 ms (0.001 min). Faster or more efficient peaks will require even better reproducibility. The above argument assumes a resolution of 1. Less resolved peaks will require more stringent t_R precision for accurate peak identification.

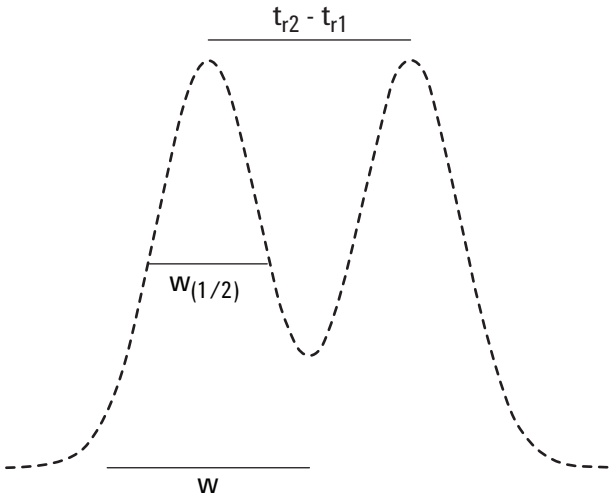

Figure 2. Example of two peaks with resolution of one and equal peak widths.

Retention time precision can also be influenced by the algorithm used by the integrator to determine the peak maximum and assign a t_R. Wider peaks (with a relatively large "flat" maximum) are the most difficult for the computer algorithms to handle accurately. Fortunately, peaks with wider peaks have larger t_R differences (for a resolution of 1), negating the need for extreme t_R precision.

Other Benefits of Extreme t_R Precision

Automated t_R Window Updating

In normal operation, a gas chromatograph performing a method will see small changes in t_R, or shifts, occur as a part of the normal aging process of the column. The difference in t_R in two sequential runs is insignificant. The difference after many runs or weeks of use will lead to the need for the t_R windows to be adjusted. This can be extremely costly to the operator (in terms of time) depending upon the number of analytes being measured. Changing the t_R window for six or less components will take less than 10 minutes. For those performing analysis with more components (such as VOA or semi-volatiles) the time requirement becomes much more substantial. Along with the cost of time the manual process adds many sources for error.

By achieving extremely good t_R precision, it is possible to update t_R windows automatically as part of the method every time the method is run. The requirement for this is identical to the previous argument or that absolute precision be better than one half the peak width so that any deviation between two subsequent runs does not result in the misidentification of the peak. Automatic updating of t_R windows is a user-settable option with the 6890N when using any Agilent data station.

Retention Time Locking (RTL)

The t_R precision is most commonly thought of as repeatability. That is the precision obtained on a single instrument, performing a particular method. For a single instrument to achieve this performance both temperature and column pressure (column flow) settings must be precise but not necessarily accurate. To achieve the same level of precision between two different instruments performing the same method requires that the same settings be accurate. That is the same temperature or pressure setting on different instruments be the same (within small tolerances) as measured by another monitoring device. The Agilent 6890N shows this type absolute accuracy. Because of this, it is possible to perform RTL on separate instruments performing the same method to produce identical (t_R) results. RTL is dependent upon the fact that the temperatures and pressures of one gas chromatograph are identical to that of another. Small differences in column length between units, which would ordinarily cause slight t_R differences, can be normalized by small changes in column pressure (flow). Benefits to the chromatographer include ease of set-up after column maintenance or replacement (no need to change t_R windows), consistent results over many years and different instruments, and the ability to directly compare results or transfer methods precisely between different laboratories.

Area Precision

Area precision of an analyte peak is the basis for all quantitative measurement in chromatography. Area precision as it relates to a GC is directly related to many aspects of the measurement. The

most important being, the ability of data processing equipment to precisely integrate signal from the gas chromatographic detector, the ability of the detector to provide the same signal for the same amount of material introduced to it and the ability to load a precise amount of sample and standard onto the chromatographic column. More than any other chromatographic measurement, area is a function of the entire chromatographic system and chromatographic method.

Area precision of the Agilent 6890N is specified at better than 1% RSD. This is specified using a splitless injection so that 2 ng of tetradecane is loaded on column and the same "generic" chromatographic conditions listed in Table 1 employed. The response of the signal is analyzed without the use of an internal standard (ISTD).

Conclusion

Area and t_R are the two primary measurements in GC. The precision with which they are measured ultimately determines the validity of quantitative data generated.

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Printed in the USA
September 16, 2005
5989-3425EN

Agilent Technologies