

PAMSGRAM

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USE OF RETENTION TIME REFERENCE PEAKS FOR PAMS

Inasmuch as the Turbochrom® software used at PAMS is relatively complex, it is no surprise that users have had difficulty with some aspects. This PAMSGram is devoted to an important area for PAMS Gas Chromatography users; a feature involving the use of chromatographic *Reference Peaks*. Although it is quite complicated, it is vital that operators understand some of Turbochrom's® nuances. This understanding will allow operators to be able to identify peaks on the chromatogram more reliably, using the built in automation features, and rely less on manual integration.

In the early days of chromatography, when gas controls were not quite as good as they are now, it was common for peaks to elute earlier or later than usual, mostly due to thermal fluctuations that affected the column carrier gas flow, so the concept of reference peaks were invented. Consider, for example, the chromatogram in Figure 1. What typically happens when system conditions change is that the peaks shift in proportion to their elution time, with the earlier peaks shifting relatively little and the later ones shifting more, as shown in Figure 2.

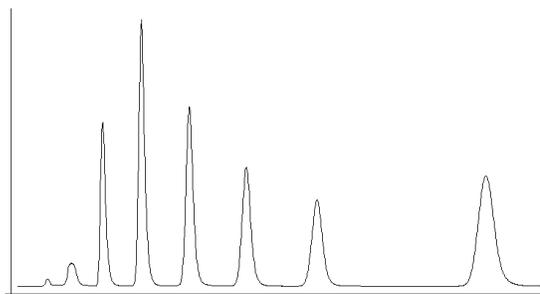


Figure 1. Standard Chromatogram Under "Normal" Conditions

There are two things to note here. The first is that earlier peaks are (usually) shifted less in absolute time terms and later peaks are (usually) shifted more (i.e., the chromatogram "stretches" from the origin or start of the run: this is clearly due to flow change). The second thing is that later-eluting peaks are usually broader than earlier peaks. This is a phenomenon of the chromatographic process and is more exaggerated for older packed columns, not the capillary columns commonly used today. A third effect which is no longer significant is a linear shift of all peaks in the chromatogram caused by a slight difference in run start time - we usually do not encounter this. To deal with these effects the programmers of the data handling software have included the following:

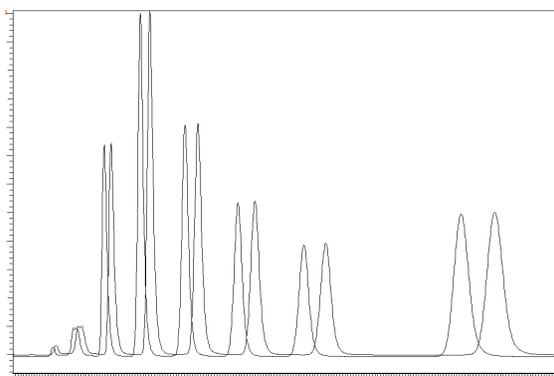


Figure 2. Time-shifted Chromatogram

1.1. **Absolute RT Window** - The *Absolute Retention Time (RT) Window* is the time in seconds during which you expect to find a peak, centered around the time for that peak set in the Data Processing Method. In Turbochrom® you can enter a different value for each peak. In order to determine the peak retention time, you would typically reprocess a whole series of data files from several hours of operation, and then find the average retention time for that peak. If you then calculate the *variation* in retention time, you can enter that value as the *Absolute RT Window*.

1.2 **Percentage RT Window** - Since the peaks are getting broader as the run progresses, you can instead enter a *Percentage Retention Time Window*, which by definition will get larger and larger (as an absolute value) as time progresses. You can enter this as a global or default value if you have many peaks to deal with so you need not enter individual values. Alternatively, you can enter the *Percent Retention Time Window* individually for each peak. The *Percent RT Window* and *Absolute RT Window* are additive.

You can see the entries for these parameters in the screen clip below (Figure 3), which you get to by selecting:

<Graphic Method Edit>
<Calibration>
<Edit>
<Components>

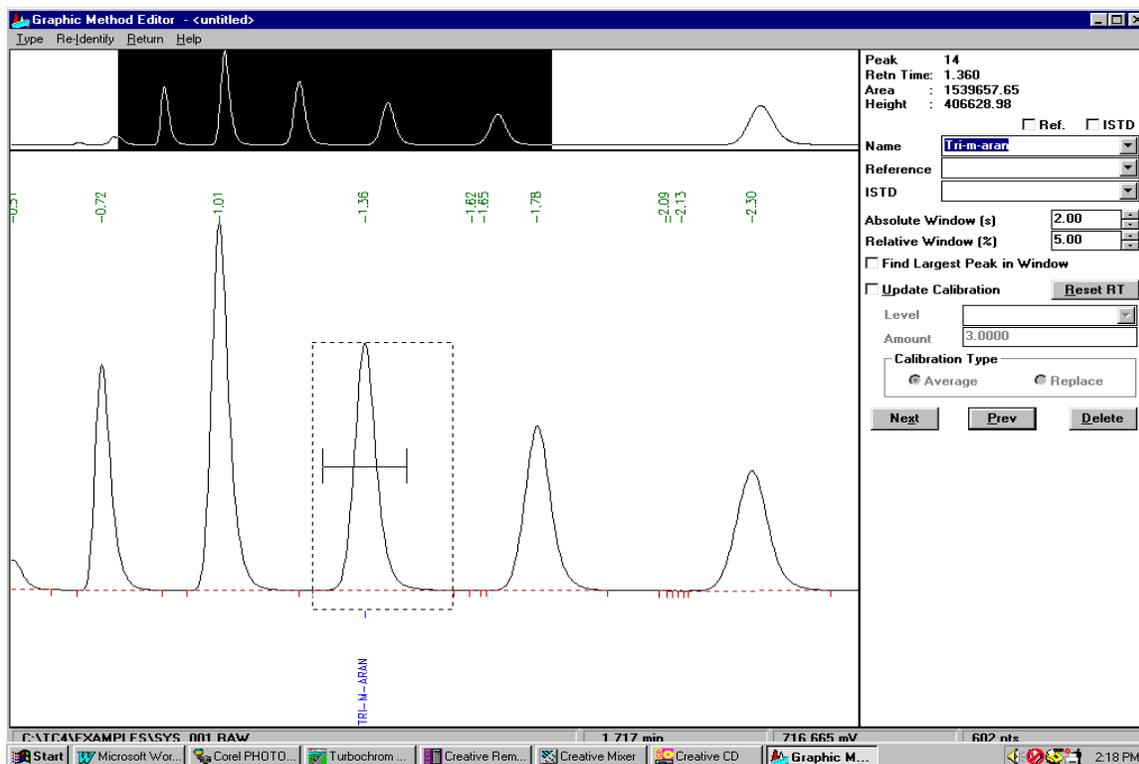


Figure 3. Graphic Method Editor Page

- 1.3. **Retention Time Reference Peak** - You can automatically adjust for some of the chromatographic peak retention time movement by defining a *Reference Peak*. This *Reference Peak* can be used to compensate for the retention time shifting of other peaks. (Note: a *Reference Peak* is a quite different concept from an Internal Standard. A *Retention Time Reference Peak* is not an Internal Standard.)

Turbochrom® software permits the designation of numerous reference peaks. Select Reference peaks with care, and in order not to complicate the matter, use as few as necessary. See Section 4.0.

2.0 DEFINITION OF TERMS

For clarification, it is important for the rest of this narrative to understand certain terms relating to the elution of chromatographic peaks.

Retention Time (RT) - This is the elution time at the crest of the chromatographic peak with respect to the start of the run. The computer calculates this as the time when the signal rate of change is zero (where data inversion has occurred).

Turbochrom Retention Time (TcRT) - In the Turbochrom® Data Processing Method a list of the compounds that you are interested in reporting are entered, along with the times that they typically elute. These TcRTs are shown schematically in Figure 4. As you can see the actual RT and the TcRT may sometimes be slightly different owing to gas flow change, which is where the peak matching "window" comes in to play. Turbochrom® has to determine which peak this really is, based on the *Retention Times* and *Retention Time Windows* you have provided for target peaks in the Data Processing Method.

Important Note: The Turbochrom® software and manual refers to the TcRT as the "Expected RT".

Expected Retention Time (ExRT) - If a reference peak is used, then this is the Turbochrom® RT corrected by the time offset of the Reference Peak. We can show this schematically (again, somewhat exaggerated for explanatory purposes) in Figure 5.

To summarize, the RT is the actual time of peak elution after the run start. The TcRT is the retention time for that compound as listed in the Turbochrom® Data Processing Method. The Expected RT is the time that Turbochrom is looking to find that peak, based on the actual movement of the *Reference Peak*.

Back to the real issue at hand.

Turbochrom® does not normally *show*

you these three pieces of data. What Turbochrom calls *Expected Retention Time* is more properly the TcRT (the time stored for that peak in the Method). In all its reports and listings, you do not normally get to see the ExRT.

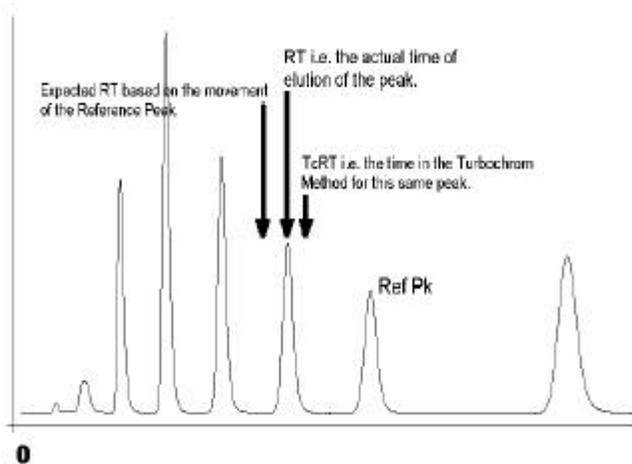


Figure 5. Expected Retention Time

factors. Since the gas controls on the GC have remarkable stability, the flow does not vary very much either. However, it is good practice to include a reference peak(s) to ensure proper peak identification in the event that system conditions do change.

The PLOT column is a different case altogether. It is severely influenced by humidity, which can be seen by the broad changes in the retention of certain compounds, particularly acetylene. So a reference peak is essential. But how does one select a *Reference Peak*? What are the criteria?

How do you choose the *Reference Peak*?

A *Reference Peak* must have the following characteristics:

- ▶ Be clearly distinguishable so that the software can find and identify it reliably.
- ▶ Be relatively well resolved from its neighbors, and of good peak shape.
- ▶ Always be present in the sample chromatograms.
- ▶ Preferably be larger than its neighboring peaks.
- ▶ The retention time should be similarly affected by system conditions as the peaks it references.

If for some reason the *Reference Peak* is NOT FOUND, it can severely impede the ability of the software to find

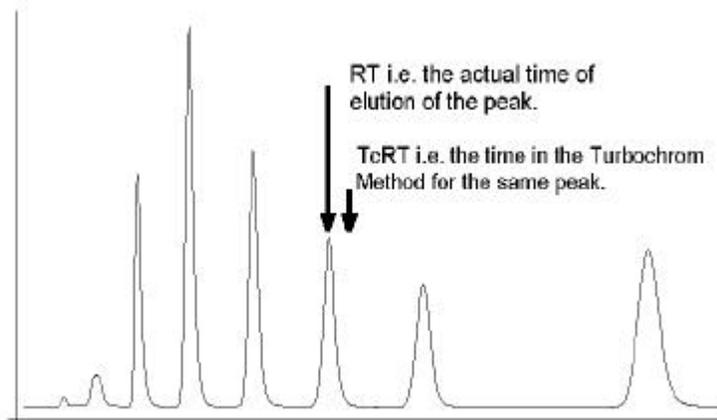


Figure 4. Retention Time (RT) and Turbochrom® Retention Time (TcRT)

3.0 SELECTION OF REFERENCE PEAKS

This Section explains how to go about selecting *Reference Peaks* and how to make the most of them to improve peak identification.

Do you need a Reference Peak?

In ambient air analysis on a PAMS system, the elution time of chromatographic peaks on the BP1 column do not vary much from run to run. A fact borne out by the thousands of hours of data that have been collected. The BP1 column is not influenced by humidity or other environmental

and identify the other peaks in the chromatogram. (This means that you must go back in after the fact and manually reprocess the data, which takes a lot of time.)

Selecting a Reference Peak on the BP1 Column

On the BP1 column you only need one *Reference Peak* since all the peak retention times are relatively stable and move in concert. This is an ideal situation and a few, well selected *Reference Peaks* throughout the chromatogram may be more desirable under certain circumstances. Toluene is recommended as a good candidate *Reference Peak* for the BP1. It meets all the criteria given above. Reference all the peaks in the chromatogram to the Reference Peak, setting the window wide enough to identify toluene reliably IN AMBIENT AIR. You may need another Data Processing Method for the calibration run, since the compounds may be present at different concentration levels from ambient air. Figure 6 shows the position of toluene in a calibration standard run, it is the 2nd peak in the quartet between 26 and 29 minutes.

Toluene is a good candidate *Reference Peak* since it is always present, does not coelute with anything, and it is the largest peak in ambient air which can accommodate a very wide RT window. Be sure to check the box "Find largest peak in the window" in the Turbochrom® Graphic Editor. You will find that all peaks move equally on the BP1 column, so it is sufficient to reference all peaks to Toluene. Having MORE *Reference Peaks* is not necessarily better, as mentioned before; always try to use the minimum number.

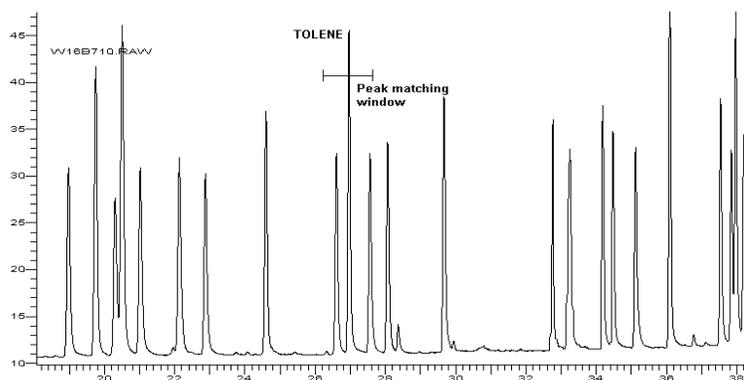


Figure 6. BP1 Column, with Toluene at 27 Minutes

Selecting a Reference Peak for the PLOT Column

Selecting a *Reference Peak* for the PLOT column is far more difficult since this column has a much higher affinity for polar species than the BP1 column and is also affected by moisture. This situation is much more interesting and therefore requires some careful thought.

The reference chromatogram is shown in Figure 7, with a dividing line drawn after acetylene. In practice the lightest molecular weight compounds move around the most in retention. The peaks are identified as follows: 1 = ethane, 2 = ethene, 3 = propane, 4 = propene, 5 = isobutane, 6 = butane. Use a *Reference Peak* for these compounds that can be readily identified and which moves around in concert with them. Butane is a good choice since it is always present. You can check that it is the largest peak in the window, and make the window quite wide.

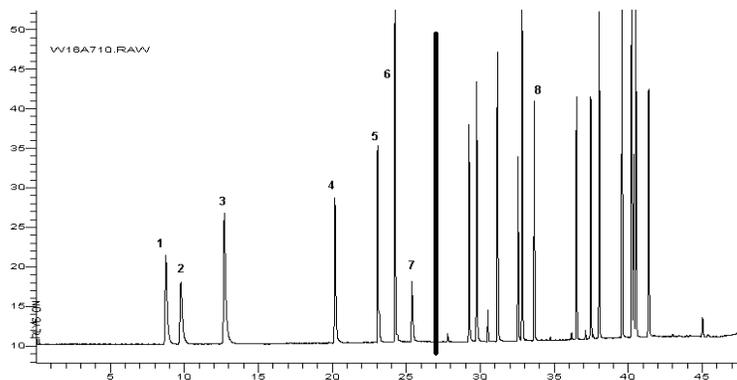


Figure 7. PLOT Column Chromatogram

Acetylene moves around the most of all of the peaks owing to humidity effects, so identify it separately and do not use a *Reference Peak*. Just set a wide peak matching window for it.

The remaining peaks follow quite an orderly routine in general, at least up to isoprene, so define a suitable *Reference Peak* for them as a group. A good choice is pentane (peak 8 in Figure 7) since it is always present and is clearly separated in time from any other compound.

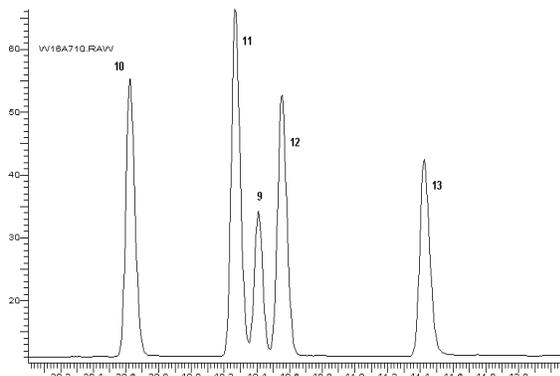


Figure 8. “The Terrible Trio” in a Calibration Standard

other compounds. The peak matching algorithm will go through the peak assignments first, looking to match *Reference Peaks*, before it starts to identify individual compounds.

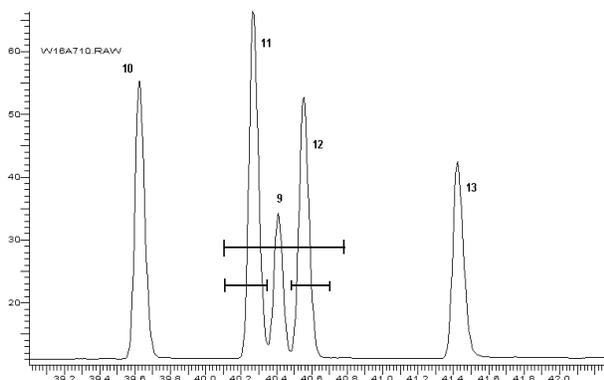


Figure 10. Setting Up Turbochrom Windows for the “Trio”

You can use the calibration standard in the beginning to develop a working Method, but the calibration standard may sometimes give misleading results since:

- ▶ It is likely that retention times will be slightly off. The retention time of a large peak is usually different because the peak crest is displaced from the smaller version of the same peak as shown in Figure 11.
- ▶ The standard is unlikely to behave like real ambient air since the humidity level will generally be different.
- ▶ The standard will not contain other (unidentified) peaks in ambient air that can cause misidentifications.
- ▶ A non-PAMS retention time standard may not necessarily contain all the target peaks. If a reference peak is missing, for example, this could cause the software to be confused.
- ▶ Signal-to-noise sensitivity values will invariably be different between the standard and the ambient sample, creating different integration results.

Finally there are three peaks that the Turbochrom® software finds difficult to identify consistently at the back end of the chromatogram as shown in Figure 8. The three peaks are peaks 11 = 2,3-dimethylbutane, 9 = 2-methylpentane, 12 = 3-methylpentane. Peak 13 is isoprene. In ambient air (Figure 10) peak 9 appears to always be present and at the highest concentration of the three. To identify these peaks more reliably, one could either designate isoprene as a *Reference Peak* and then reference the trio to it, or find the central peak and use it as a *Reference Peak*. The way to do this would be as shown in Figure 9 for an ambient sample. Define a wide window for the *Reference Peak* and smaller windows for the

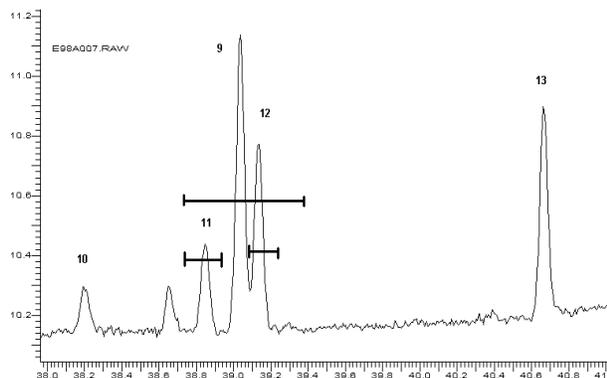


Figure 9. The “Terrible Trio” in Ambient Air

Figure 10 gives an example of the windows for a calibration standard so you can see the rationale behind the checking the settings for the windows using ambient air.

As long as 2-methylpentane (peak 9) is the largest peak in this group of three, this arrangement will work well. You can also see from this that it can be difficult to develop a good Turbochrom® Data Processing Method for ambient air from a high level calibration standard.

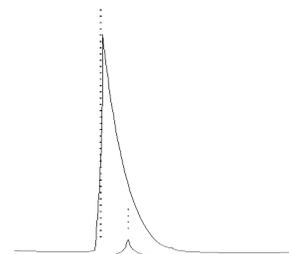


Figure 11. Large Peak versus Small Peak Crest

It is suggested to have a separate Data Processing Method altogether for the calibration standard run. Concentration levels and characteristics of the calibration standard can be significantly different from those found in the ambient air, therefore two Data Processing Methods are recommended.

4.0 CONCLUSIONS

- ▶ With an understanding that *Reference Peaks* have important characteristics, select the minimum number to accomplish your goals.
- ▶ Do not assume that the same Data Processing Method that works on ambient air will work for a calibration standard.
- ▶ You should not have to re-process chromatograms manually to identify peaks. If you are presently doing this, then your Data Processing and Acquisition Methods may not be optimized.

A subsequent PAMSGram will address the topic of setting retention time windows in Turbochrom®.