Volume 16

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Using Turbochrom® Software

INTRODUCTION

In ***PAMSGram*** #14, we discussed some of the issues concerning selecting retention time reference peaks and peak identification in Turbochrom® and how this might be applied to the PAMS chromatogram. So, in this ***PAMSGram*** we will go much further and actually go through development and optimization of a method so that it is robust. There may be some repetition from the previous ***PAMSGram*** but it will be worthwhile. The whole idea is that we have a computer in front of us, so why not make it do more of the work? It seems to be counterproductive to spend additional hours refining, reintegrating and generally massaging the data until we are satisfied. This leads to a data backlog or bottleneck.

GETTING STARTED

The **first** thing to do is let the system come to operating equilibrium for at least a week or ten days. This is particularly important for the PLOT column stability; the background signal from the BP-1 column will also slowly come down over time. The *second* thing to do is to acquire some data.

Let us consider acquiring data. We obviously do not start by looking at scores of hourly ambient air runs, because they probably do not contain all the compounds in our target list. So we need something else.

The standard that is supplied through the EPA contains all the requisite compounds but it is DRY! Since the single biggest cause of retention time variation in this system is humidity, it really makes sense to try to use a standard that is as close to ambient air in humidity content as possible. (There are other reasons, such as carryover problems.) In order to get several reproducible runs, we need to have on hand a properly humidified canister standard, roughly 10 to 40 ppbC, at about 60% to 70% relative humidity (RH).

Further, since we would like multiple runs of this standard, it makes sense to use a 33-liter canister if available, with a starting pressure of roughly 30 psi gauge. You will be removing about 600 cc of standard for each run; actually closer to 900 cc when you count what is used for purge gas, etc. It is practical to use a 33-liter canister, but a 6-liter canister works; just ensure that the pressure does not drop below the point of usability (5 psig is the minimum to use).

Once armed with a properly humidified retention time standard, perform a number of runs. Do not use a reference peak at all. For this first set of data you need to do this the hard way. We will use as our first example some data that was obtained recently at a new site in the Midwest. The Peak Summary results files are first output as a .CSV file summary and imported into Microsoft Excel®, as shown in this example; data can be entered manually instead:

Table 1 - Sample Summary Data Excerpted from Turbochrom®

	43220 N-PENTANE		43226 TRANS-2-PENTENE		43224 1-PENTENE		43227 CIS-2-PENTENE	
File Name	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]
MUL914b1.rst	28.855	91840	31.76	89707	32.5	89271	33.193	124203
MUL914b2.rst	30.98	85949	34.02	103283	34.98	83929	35.57	116562
MUL914b3.rst	31.505	84555	34.552	83345	35.553	82623	36.129	114212
MUL917b1.rst	29.59	89638	32.54	87234	33.37	86612	34.04	120537
MUL917b2.rst	30.97	85959	34.01	85815	34.98	82808	35.57	115050
MUL917b3.rst	31.49	83664	34.55	82602	35.55	83334	36.13	113710
MUL917b4.rst	31.665	83712	34.733	85966	35.75	91823	36.32	114037
MUL915b1.rst	30.78	90032	33.78	88292	34.72	88102	35.34	121104
MUL915b2.rst	31.26	85063	34.29	84931	35.27	83355	35.85	114467
MUL-D22b.rst	31.66	47249	34.74	47240	35.74	46216	36.338	64239
MUL-106b.rst	31.63	45360	34.69	45411	35.69	44966	36.28	62537
MUL-D23b.rst	31.67	46824	34.76	47765	35.76	46044	36.35	64501
MUL-N23b.rst	31.32	54216	34.4	53131	35.38	52101	35.97	73232
MUL-D02b.rst	31.33	51355	34.396	50265	35.37	49256	35.964	68708
Averages	30.76	96413	33.72	48547	34.71	63534	35.33	49686
%RSD	1.5	24	2.03	121	1.53	48	1.37	120

Table 1 is extracted from a typical Peak Summary result file from Turbochrom®. Now assuming that the method parameters have been set properly, the data in the table will be correct. You might think it is fairly easy to spot when something is wrong, but some errors can be subtle and the table should be thoroughly checked, especially the first time. Here are some examples of how the file should NOT look, starting with **Table 2.**

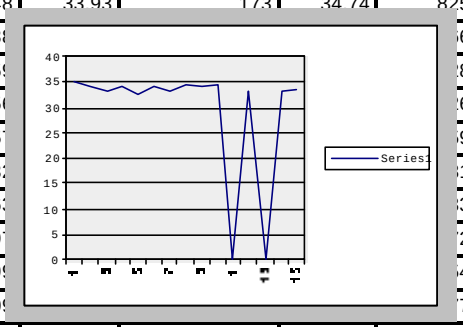
Table 2 - Example of a Problematic Summary Entry

	43220 N-PENTANE		43226 TRANS-2-PENTENE		43224 1-PENTENE		43227 CIS-2-PENTENE	
File Name	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]
MUL914b1.rst	31.76	89707	35.2	177678	35.88	130	36.32	132072
MUL914b2.rst	30.98	85949	34.02	103283	34.98	83929	35.57	116562
MUL914b3.rst	30.63	133162	33.14	558	34.55	83346	35.17	1240
MUL914b4.rst	30.81	134848	33.93	173	34.74	82591	35.41	17595
MUL917b1.rst	29.59	89638	32.54	87234	33.37	86612	34.04	120537
MUL917b2.rst	30.97	85959	34.01	85815	34.98	82808	35.57	115050
MUL917b3.rst	30.61	132556	33.15	1104	34.55	82602	35.17	1268
MUL917b4.rst	30.79	131857	34.2	1580	34.73	85966	35.36	1361
MUL915b1.rst	30.78	90032	33.78	88292	34.72	88102	35.34	121104
MUL915b2.rst	31.26	85063	34.29	84931	35.27	83355	35.85	114467
MUL-D22b.rst	30.77	74897	0	0	34.74	47240	35.38	635
MUL-106b.rst	30.74	72699	33.34	332	34.69	45411	35.33	727
MUL-D23b.rst	30.78	74709	0	0	34.76	47765	35.39	593
MUL-N23b.rst	30.43	84751	33.04	117	34.4	53131	35.03	1054
MUL-D02b.rst	30.44	80366	33.67	21	34.29	20	35.02	1022
Averages	30.76	96413	33.72	48547	34.71	63534	35.33	49686
%RSD	1.5	24	2.03	121	1.53	48	1.37	120

You can see that there is a problem here with trans-2-pentene because the time and area are both zero, in other words the peak is not found. The file must be examined to determine why this happened and the problem resolved; then reprocess the data and run the Peak Summary again. Another way of finding a problem of this kind is to plot the data in Excel® or VOCDat. An example of a spreadsheet (Excel®) and time series plot (VOCDat) is shown in **Table 3.**

Table 3 - Another Example of a Problematic Summary Entry

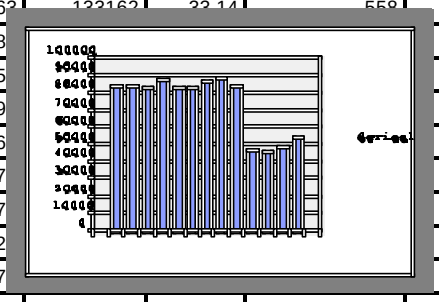
	43220 N-PENTANE		43226 TRANS-2-PENTENE		43224 1-PENTENE		43227 CIS-2-PENTENE	
File Name	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]
MUL914b1.rst	31.76	89707	35.2	177678	35.88	130	36.32	132072
MUL914b2.rst	30.98	85949	34.02	103283	34.98	83929	35.57	116562
MUL914b3.rst	30.63	133162	33.14	558	34.55	83346	35.17	1240
MUL914b4.rst	30.81	134848	33.93	173	34.74	82591	35.41	17595
MUL917b1.rst	29.59	8963				612	34.04	120537
MUL917b2.rst	30.97	8595				808	35.57	115050
MUL917b3.rst	30.61	13255				602	35.17	1268
MUL917b4.rst	30.79	13185				966	35.36	1361
MUL915b1.rst	30.78	9003				102	35.34	121104
MUL915b2.rst	31.26	8506				355	35.85	114467
MUL-D22b.rst	30.77	7489				240	35.38	635
MUL-106b.rst	30.74	7269				411	35.33	727
MUL-D23b.rst	30.78	7470				765	35.39	593
MUL-N23b.rst	30.43	84751	33.04	117	34.4	53131	35.03	1054
MUL-D02b.rst	30.44	80366	33.67	21	34.29	20	35.02	1022
Averages	30.76	96413	33.72	48547	34.71	63534	35.33	49686
%RSD	1.5	24	2.03	121	1.53	48	1.37	120



The irregularities in the trans-2-pentene data are an instant flag that something is wrong. The %RSD values and "Averages" do not provide obvious data to flag this condition; it is up to you to search for these artifacts and fix them. Another way of spotting problems is by plotting the area data as shown for 1-pentene in **Table 4.**

Table 4 - Example of Problematic Area Data

	43220 N-PENTANE		43226 TRANS-2-PENTENE		43224 1-PENTENE		43227 CIS-2-PENTENE	
File Name	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]
MUL914b1.rst	31.76	89707	35.2	177678	35.88	130	36.32	132072
MUL914b2.rst	30.98	85949	34.02	103283	34.98	83929	35.57	116562
MUL914b3.rst	30.63	133162	33.14	558	34.55	83346	35.17	1240
MUL914b4.rst	30.8				34.74	82591	35.41	17595
MUL917b1.rst	29.5				33.37	86612	34.04	120537
MUL917b2.rst	30.9				34.98	82808	35.57	115050
MUL917b3.rst	30.6				34.55	82602	35.17	1268
MUL917b4.rst	30.7				34.73	85966	35.36	1361
MUL915b1.rst	30.7				34.72	88102	35.34	121104
MUL915b2.rst	31.2				35.27	83355	35.85	114467
MUL-D22b.rst	30.7				34.74	47240	35.38	635
MUL-106b.rst	30.74	72699	33.34	332	34.69	45411	35.33	727
MUL-D23b.rst	30.78	74709	0	0	34.76	47765	35.39	593
MUL-N23b.rst	30.43	84751	33.04	117	34.4	53131	35.03	1054
MUL-D02b.rst	30.44	80366	33.67	21	34.29	20	35.02	1022
Averages	30.76	96413	33.72	48547	34.71	63534	35.33	49686
%RSD	1.5	24	2.03	121	1.53	48	1.37	120



In this example the first and last data points for 1-pentene are obviously in error. So even though the retention time looks OK, the area data are inconsistent and this shows that the wrong peak has been identified. Of course, one way to minimize this in the first place is to use a value for area reject that will eliminate some of these misidentifications.

Incidentally, you can see from this data set that something happened to affect the recovery of this and all other compounds starting with file MUL-D22b.rst. This bears investigation separately (for the purposes of RT evaluation this may not be a problem).

Now you have a valid data set, with all the peaks found and listed properly. (Do not be surprised if this takes a bit of effort) What you now need to determine is "what is the normal time that I would expect each component to elute?"

FINDING THE CORRECT ELUTION TIME

The time that we want for the elution time of a peak is the mid-point of the extremes of the range of values. Let us look at a diagram of this to get a clear mental picture. In **Figure 1**, each X represents one run, so we are looking at approximately 35 runs, which are distributed in time between T1 and T2 (Time 1 and Time 2). T1 is the lowest or earliest elution value we have, and T2 is the highest or latest elution value.

All of the runs fall between these two, and the distribution falls roughly in the shape of the curve. The **MODE** or most frequent result is found at the top of the curve. The **MEDIAN** is shown, which is the point where half of the result falls on each side. The **AVERAGE** or **MEAN** will be somewhere near the median.

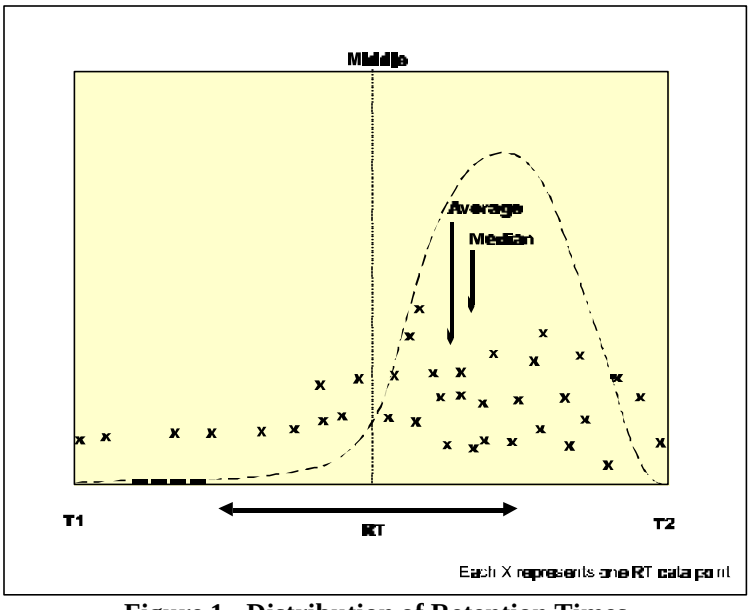


Figure 1 - Distribution of Retention Times

In Turbochrom® we are given two parameters to set, the **Retention Time** and the **WINDOW.** You can probably see that the retention time value we want for Turbochrom® is the **MIDDLE** of this set of data, and the window is the difference between T1 and T2, i.e. T2 minus T1. If your data are superb, and the retention times are within close tolerances, it is quite satisfactory to use the **median** value, which is the point at which half of the observations fall on each side. The **average** is really of no use, since it is more related to the *absolute values* of the observations rather than their *relationships*. There is no statistical function in Excel® to easily provide what is needed; it can be calculated directly in the spreadsheet table. Add another three rows to the Excel® file as shown in **Table 5.**

Table 5 - Calculating the Middle RT and Span

	43220 N-PENTANE		43226 TRANS-2-PENTENE		43224 1-PENTENE		43227 CIS-2-PENTENE	
File Name	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]	Time [min]	Area [µV·s]
MUL914b1.rst	28.855	91840	31.76	89707	32.5	89271	33.193	124203
MUL914b2.rst	30.98	85949	34.02	103283	34.98	83929	35.57	116562
MUL914b3.rst	31.505	84555	34.552	83345	35.553	82623	36.129	114212
MUL917b1.rst	29.59	89638	32.54	87234	33.37	86612	34.04	120537
MUL917b2.rst	30.97	85959	34.01	85815	34.98	82808	35.57	115050
MUL917b3.rst	31.49	83664	34.55	82602	35.55	83334	36.13	113710
MUL917b4.rst	31.665	83712	34.733	85966	35.75	91823	36.32	114037
MUL915b1.rst	30.78	90032	33.78	88292	34.72	88102	35.34	121104
MUL915b2.rst	31.26	85063	34.29	84931	35.27	83355	35.85	114467
MUL-D22b.rst	31.66	47249	34.74	47240	35.74	46216	36.338	64239
MUL-106b.rst	31.63	45360	34.69	45411	35.69	44966	36.28	62537
MUL-D23b.rst	31.67	46824	34.76	47765	35.76	46044	36.35	64501
MUL-N23b.rst	31.32	54216	34.4	53131	35.38	52101	35.97	73232
MUL-D02b.rst	31.33	51355	34.396	50265	35.37	49256	35.964	68708
Min =min(Cell1 .. Cell2)	28.855		31.76		32.5		33.193	
Max =max(Cell1 .. Cell2)	31.67		34.76		35.76		36.35	
Middle =((max - min)/2)+min	30.2625		33.26		34.13		34.7715	
Span =max - min	2.815		3		3.26		3.157	
Averages	30.76	96413	33.72	48547	34.71	63534	35.33	49686
%RSD	1.5	24	2.03	121	1.53	48	1.37	120

So here we have inserted additional rows and calculated the **min**imum, **max**imum, **middle** and **span** (difference between the max and min values). The functions used to calculate these values are shown in the first cell. It is easier than it looks to do this in Excel®. First you create space (more rows) in which to insert the functions, then type the functions into the appropriate cells. Use "block select" to pick the range of cells you want. Then copy these cells to the next column where they are needed and so on across the page. Then block select and paste these values in all equivalent positions down the chart and they will fill in the correct data automatically. If you have never done it before you should practice on a dummy sheet. If in doubt, type in the entries.

The result of all of this is that you now have the **MIDDLE** values for each of the components. These are the values that must go into Turbochrom® for TIME (**Figure 2**). This is where we start to have difficulty because, as we stated in ***PAMSGram*** 14, Turbochrom® incorrectly uses this term to mean "expected retention time". To be consistent with the earlier ***PAMSGram*** we will use the term **TcRT** for Turbochrom® RT. Further, you cannot use the Graphical Method Editor in Turbochrom® for this. Go into Turbochrom® and select the BUILD METHOD button. Open the PROCESS section of the method and go into the Components area. You must modify each component individually, putting in the correct MIDDLE value for the "Retention" value. It is easier and "safer" to input this information in the method builder rather than the Graphical Method Editor.

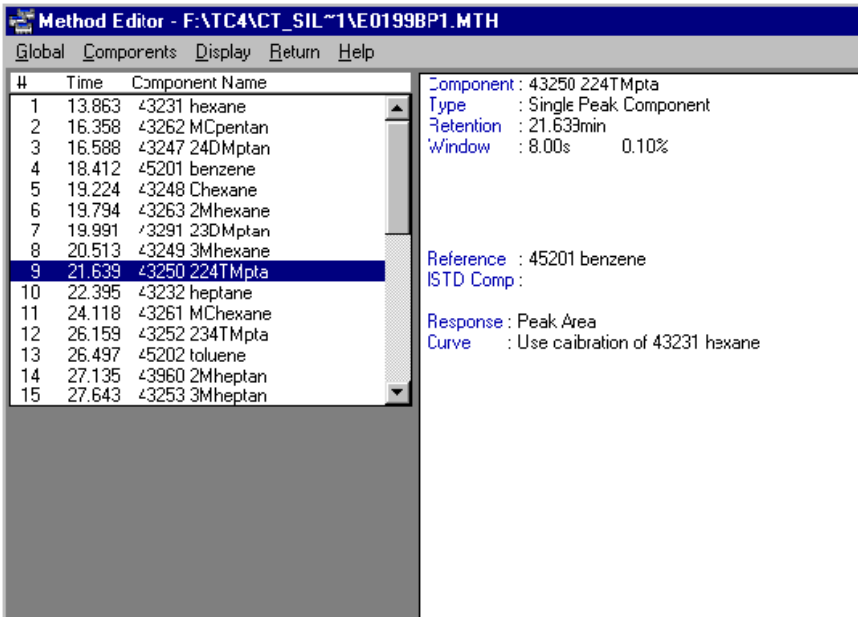


Figure 2 - Editing the Method

Also in this dialogue box you can set the "Abs Window" and "Rel Window" (**Figure 3**). As discussed in ***PAMSGram*** #14, we do not need a Rel Window because our peaks are not getting broader throughout the run, so set this to 0%. We cannot properly set the Abs window yet,

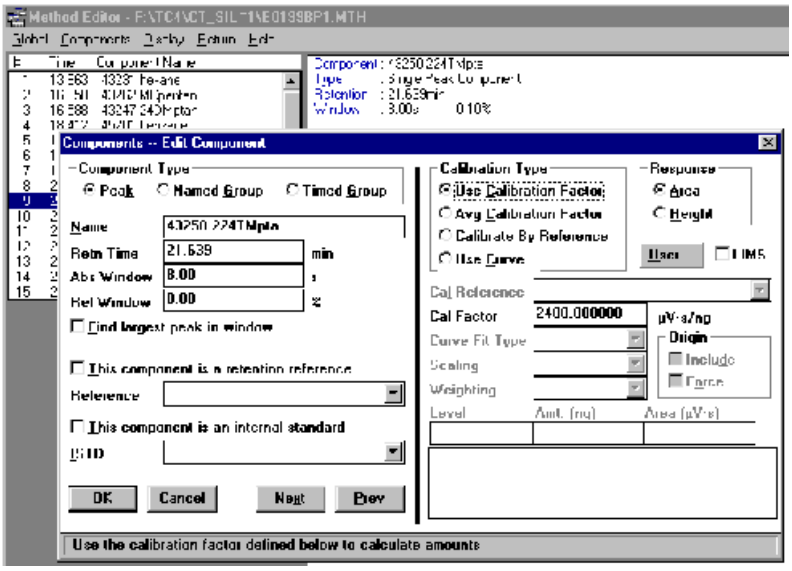


Figure 3 - Setting the Windows

because we do not have enough data. It will be sufficient to set this to 5 seconds for now, which equates to plus and minus 2.5 seconds around our **MIDDLE** value. Obviously this is not going to be correct but it is easy to reset it later.

There is actually a better way of doing this, by setting the value under COMPONENT DEFAULTS, as shown in **Figure 4.** This enables you to set the values once and they will be inserted properly by default for the rest of the compounds. After this process is finished you should have a good components list with all of the components set to the MIDDLE retention times.

SELECTING A REFERENCE PEAK

Now let us look at the BP-1 chromatogram and figure out how to deal with retention time shifts.

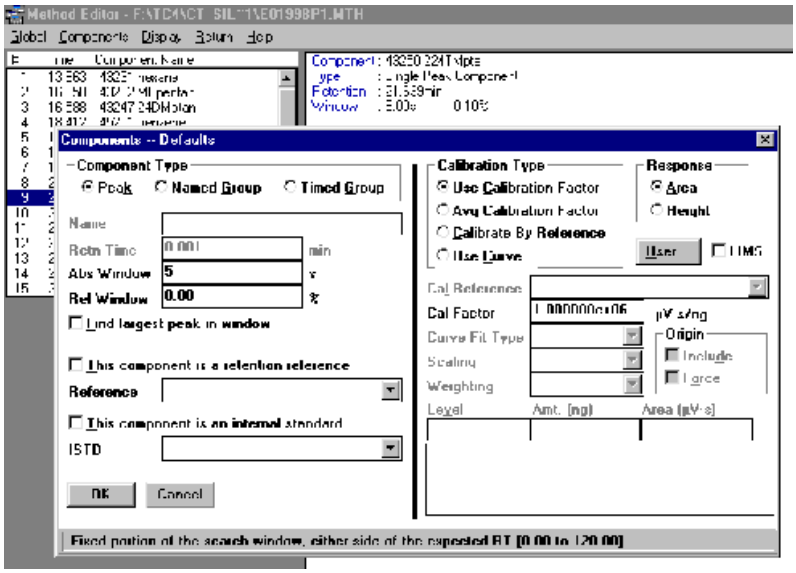


Figure 4 - Setting Default Values

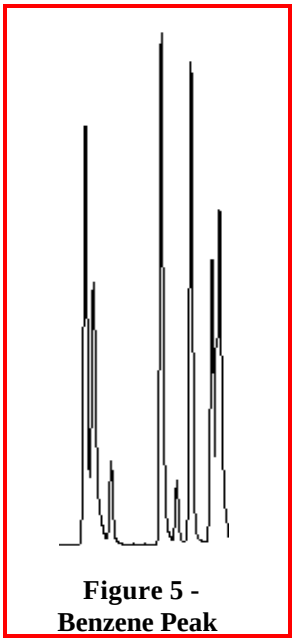


Figure 5 - Benzene Peak

In order to deal with shifting retention times the programmers of the data system have given us a parameter called the "Reference Peak". As was discussed in the earlier ***PAMSgram*** #14, the Reference Peak should have certain attributes or qualities:

1. It should be clearly distinguishable from other peaks so the software can find it,
2. It must be relatively well resolved from its neighbors and of good peak shape,
3. It must always be present in every run,
4. It should preferably be larger than neighboring peaks,
5. Its retention should be affected similarly to the peaks it references.

In ambient air there are two peaks immediately come to mind, toluene and benzene. Let us look at these two in the next two figures. **Figure 5** shows benzene (the tallest peak), and just to its right is a small peak, which is a chlorinated hydrocarbon, which is not on the target list. There is a very real possibility that the artifact peak could be actually larger than benzene in some circumstances. If the canister standard was contaminated or there was a source of some sort nearby, we could mistake this peak for benzene and lose our peak matching, so benzene is not our first choice for a reference peak.

The second detail in **Figure 6** shows toluene (the largest peak). In ambient air there is nothing that ever elutes close to toluene or exceeds it in concentration, so toluene is our pick for reference peak. Further, since toluene is always the largest peak in this group, we can use another feature of the software - "Use largest peak in the window". Let us see how that works.

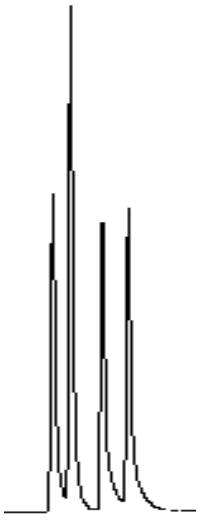


Figure 6 - Toluene Peak

Notice that the box "Find largest peak in the window" is checked. Set the window to 30 seconds then SAVE the method.

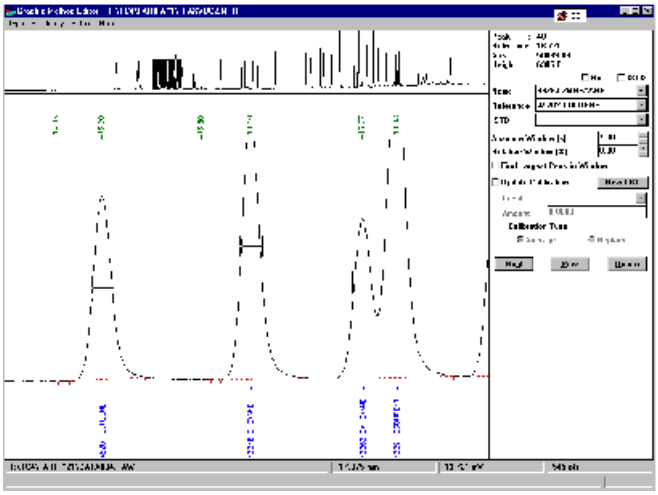


Figure 7 - Are the Peaks Matched?

Now go into graphical method editor (GME) and pull up the method and look at one of your calibration standards. If all is well you will see that all the peaks are properly matched. The way to tell this is by looking at the dumbbell (horizontal line with a vertical at each end) in the Edit Components window. If the peak is matched, the dumbbell will be halfway up the peak; if it is not, the dumbbell will be down on or below the baseline. The accompanying **Figure 7** shows a good example of properly matched peaks.

However, the biggest problem with the whole process is that Turbochrom® sometimes misleads you! Not deliberately of course, but it is all related to the issue of "expected" retention times. In the next example, you could think that Turbochrom® has misidentified the peaks. It has not; it is just putting up the dumbbell at what it calls the "expected" retention time of each peak. In fact, it is the retention time for that peak as listed in the method, with the dumbbell shown.

The clue is that the dumbbell is above the baseline, meaning that something is matched, but you are not sure what.

In the detail from GME in **Figure 8**, you can see an example of the BIG problem (this is a deliberately exaggerated example). The dumbbells are not on the peaks. They are drawn at the TcRT (the time stored for each peak in the method). If you saw this situation, would you not adjust the retention times? WRONG MOVE!

To see where Turbochrom® is *really* looking to identify these peaks you must put the cursor on your reference peak (in this case the customer used benzene) and click on **Update RT.** Now the dumbbells will jump to the real EXPECTED RETENTION TIMES (**ExRT)** shown in **Figure 9.**

When you click on the *reference peak* and select Update RT the dumbbells go to their new positions (ExRT) and are displayed in red. Only the few component peaks associated with that reference peak are affected; if you have more than one reference peak, then other peaks will not be affected by this action and they will not go red. At this point DO NOT SAVE THE METHOD!!!!! If you do, you will modify the retention time of the reference peak. The reference peak is located at the MIDDLE of the range window as you recall. This particular run may be on the edge of the envelope (i.e., an extreme result, or atypical) and you certainly would not want to re-define your results on the basis of an atypical result.

This is the whole idea behind method development. You want to create a method that is normal (i.e., it is centered about the range of possible results, yet it is able to deal with the swings in the retention results automatically). The way to do this is not to use GME to *edit* the method, but use GME to *view* the results.

An important point worth noting, there is a bug in some versions of this software - once you have viewed the data, **exit GME immediately**. Do not pull up another file, because the results will be displayed in red even though they should be displayed in black, so you will not be sure what you are looking at. Exit immediately! To view another file, re-start GME from the Turbochrom® Navigator beginning screen.

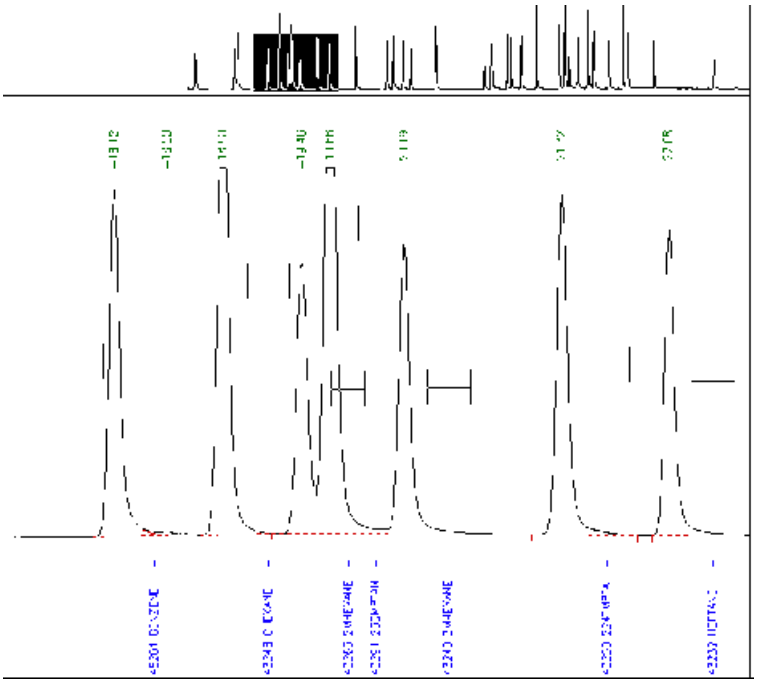


Figure 8 - Turbochrom Peak Matching

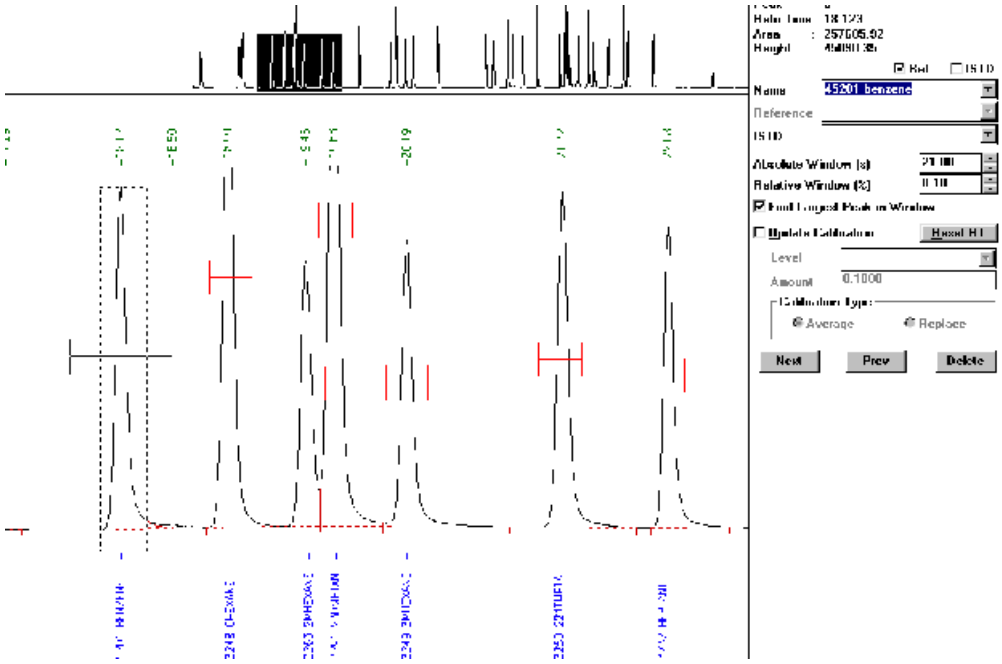


Figure 9 - Actual Expected Values In Red

APPLYING YOUR NEW METHOD TO REAL DATA

Now that you have your method developed, you can apply it to real data. Pull up an ambient run and look to see that the peaks are being identified as you expected. There may be some small differences, since your initial development was done on a calibration standard, but you should be able to refine your method over time to improve its performance. The main thing is to make subtle changes of a few seconds or so to the data. Do not use the Graphical Method Editor to make changes, since that will simply move the middle position that you have worked so hard to establish.

THE PLOT COLUMN

Here are some data, manually extracted from the records of approximately two hundred runs:

Table 6 - PLOT column data

Compound	RT Max	RT Min	RT Middle	RT Span
ethane	7.95	7.91	7.93	0.04
Ethylene	8.75	8.65	8.7	0.1
Propane	11.13	10.98	11.055	0.15
Propylene	18.44	17.96	18.2	0.48
Isobutane	21.28	20.91	21.095	0.37
n-Butane	22.47	22.14	22.305	0.33
Acetylene	24.46	23.92	24.19	0.54
Trans-2-butene	27.47	27.16	27.315	0.31
1-Butene	28.05	27.7	27.875	0.35
Isobutene	28.78	28.42	28.6	0.36
cis-2-butene	29.42	29.09	29.255	0.33
cyclopentane	30.82	30.52	30.67	0.3
isopentane	30.95	30.65	30.8	0.3
n-pentane	31.75	31.44	31.595	0.31
2-me-2-butene	34.36	34.07	34.215	0.29
cyclopentene	34.38	34.19	34.285	0.19
trans-2-pentene	34.64	34.31	34.475	0.33
3-me-1-butene	35.29	34.97	35.13	0.32
1-pentene	35.61	35.35	35.48	0.26

cis-2-pentene	36.13	35.93	36.03	0.2
2,2-dimetbutane	37.65	37.4	37.525	0.25
2,3 dimethylbutane	38.33	37.98	38.155	0.35
2-mepentane	38.44	38.13	38.285	0.31
3-mepentane	38.6	38.26	38.43	0.34
isoprene	39.62	39.25	39.435	0.37
4-methyl-1-pentene	41.4	40.89	41.145	0.51
1-hexene	41.86	41.67	41.765	0.19

(This list is taken from some early data so accuracy cannot be guaranteed relative to today's standard.)

The interesting aspects of these data can be seen in the following plot of retention time variation:

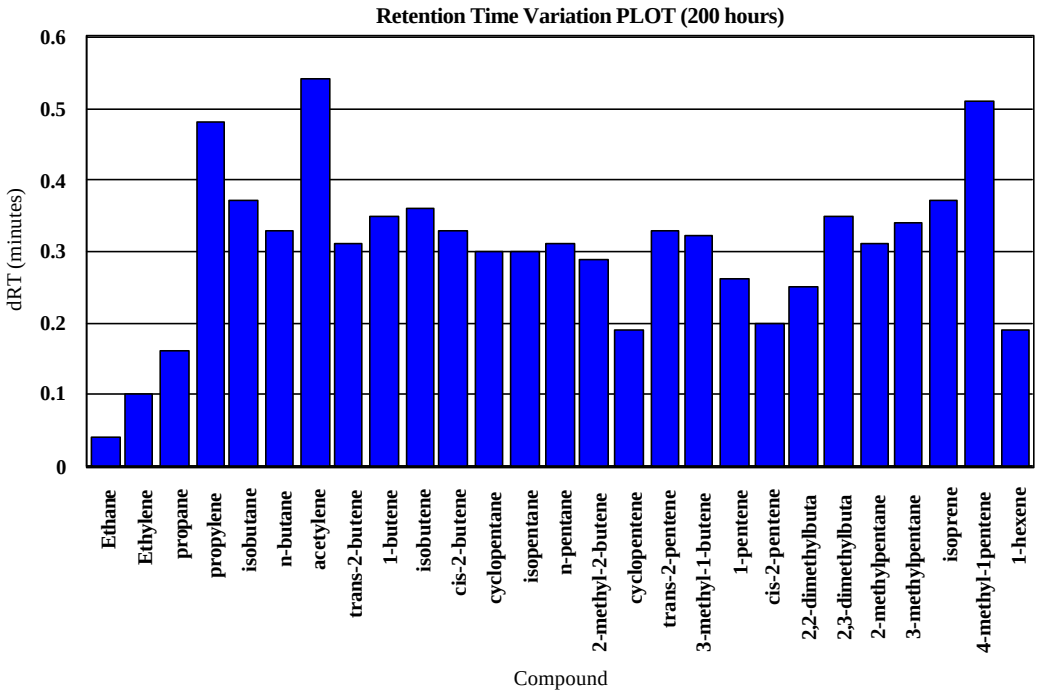


Figure 10 - PLOT Retention Variation

There are a few features to be seen in these data:

1. Hexene and the peak just before it are probably being affected by a couple of outlying data points that are throwing off the results. These should be found and considered carefully. Are they real or artifacts (misidentifications)? This is an area where contamination from polar compounds can be an issue.

2. Isobutane and acetylene have the greatest variation. This is the general experience. They are obviously atypical and therefore will probably not imitate the movement of any reference peak. They are "independent".

3. The earliest peaks have small variations, as shown.

4. Apart from these, most peaks in the chromatogram seem to be in fairly good agreement, moving roughly 2.5 to 3.5 (tenths of a minute).

So we can speculate that if we can define a single reference peak for the peaks in the main portion of the chromatogram, and it moves by ~0.3 minute, then all the peaks that also move by ~0.3 minute should also be found. Let us look at this more closely. Refer to the chromatogram in **Figure 11.**

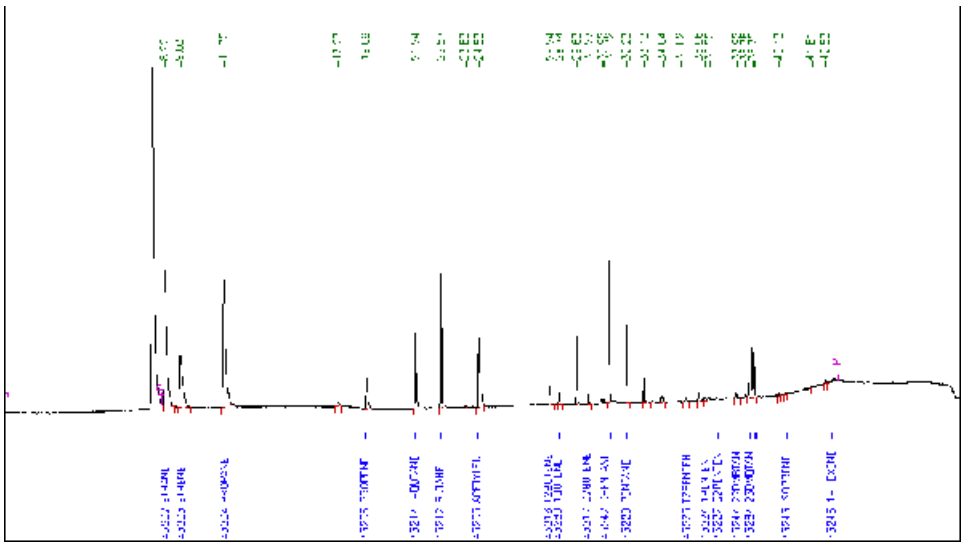


Figure 11 - The PLOT Chromatogram

What we can do next is calculate the difference between our candidate reference peak and each prospective "mate" to see if our hypothesis holds up. In **Table 7** we have assumed that pentane is a good candidate reference peak, and have calculated the difference between it and each of the later peaks in the chromatogram.

Table 7 - Retention difference relative to pentane for selected peaks.

Compound	Max	Min	Middle	Span	Span, in Seconds	Diff relative to pentane
Ethane	7.95	7.91	7.93	0.04	2.4	N/a
Ethylene	8.75	8.65	8.7	0.1	6	N/a
Propane	11.13	10.98	11.055	0.15	9	N/a
Propylene	18.44	17.96	18.2	0.48	28.8	N/a
Isobutane	21.28	20.91	21.095	0.37	22.2	N/a
n-Butane	22.47	22.14	22.305	0.33	19.8	N/a
Acetylene	24.46	23.92	24.19	0.54	32.4	N/a
Trans-2-butene	27.47	27.16	27.315	0.31	18.6	0.00
1-Butene	28.05	27.7	27.875	0.35	21	2.40
Isobutene	28.78	28.42	28.6	0.36	21.6	3.00
cis-2-butene	29.42	29.09	29.255	0.33	19.8	1.20
cyclopentane	30.82	30.52	30.67	0.3	18	-0.60
isopentane	30.95	30.65	30.8	0.3	18	-0.60
n-pentane	31.75	31.44	31.595	0.31	18.6	0.00
2-me-2-butene	34.36	34.07	34.215	0.29	17.4	-1.20
cyclopentene	34.38	34.19	34.285	0.19	11.4	xxxx
trans-2-pentene	34.64	34.31	34.475	0.33	19.8	1.20
3-me-1-butene	35.29	34.97	35.13	0.32	19.2	0.60
1-pentene	35.61	35.35	35.48	0.26	15.6	-3.00
cis-2-pentene	36.13	35.93	36.03	0.2	12	xxxx
2,2-dimethylbutane	37.65	37.4	37.525	0.25	15	-3.60
2,3 dimethylbutane	38.33	37.98	38.155	0.35	21	2.40
2-methylpentane	38.44	38.13	38.285	0.31	18.6	0.00
3-methylpentane	38.6	38.26	38.43	0.34	20.4	1.80
isoprene	39.62	39.25	39.435	0.37	22.2	3.60
4-methyl-1-pentene	41.4	40.89	41.145	0.51	30.6	-
1-hexene	41.86	41.67	41.765	0.19	11.4	-
					min	-3.60
					max	3.60
					i.e.: total	0.12 minutes

You can see that we have modified the table to calculate the actual time in seconds of the amount of variation relative to our potential reference peak n-pentane. Use pentane as the reference peak, setting the window to 30 seconds (i.e., plus/minus 15 seconds)

Looking at the table again, two obvious outliers have been eliminated (cyclopentene and cis-2-pentene), because their values were really extreme. This is probably a perfectly valid thing to do since there may have been a misidentification, but you will not know until you reanalyze the data using the new values. This is not an instant process. You will need to reanalyze and modify or fine tune the result until things fit.

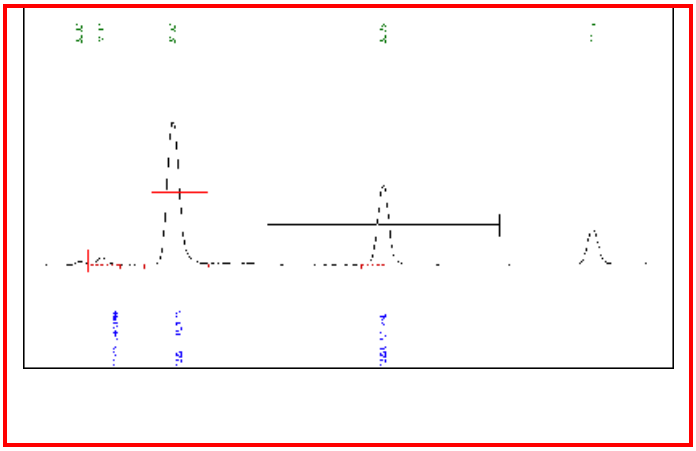


Figure 12 - A 30-Second Window on Pentane in Ambient Air

You can see from this actual air sample in **Figure 12**, that nothing elutes very close to pentane, so it is an excellent candidate for a reference peak as long as the other peaks follow its movement closely.

From the table the result is that all the peaks of interest vary by plus or minus 3.6 seconds relative to pentane. This independent movement (7.2 seconds total or 0.12 minutes) is the initial value for the window we will use for each of these peaks. The other peaks (ethane, ethylene, propane, propylene, isobutane, –butane and acetylene) are sufficiently well resolved that they can have much wider windows without being confused with any other peak. Let us look at that section in the next chromatogram (**Figure 13**).

Here the windows for ethane and ethylene are 15 seconds each, and the other windows are 30 seconds. No reference peaks are used and the largest peak in each window is found appropriately. This is ambient air.

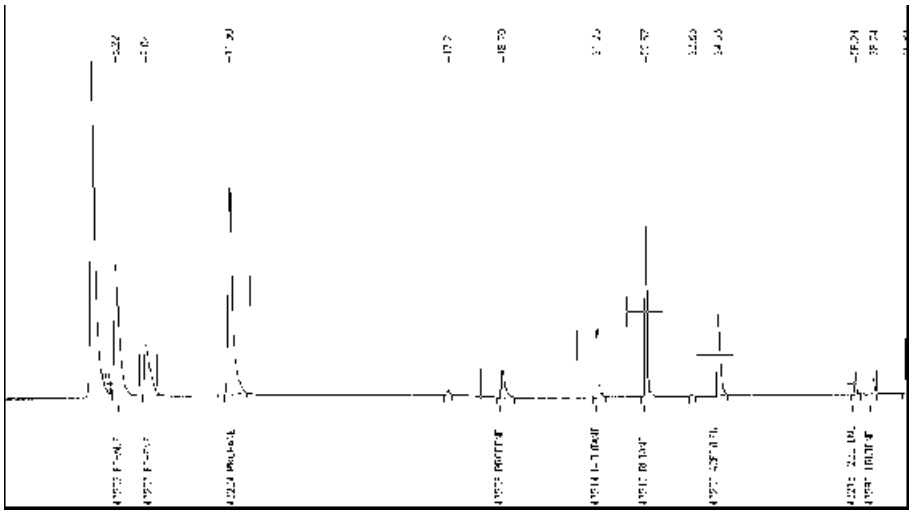


Figure 13 - The Early PLOT Compounds

SUMMARY

1. Set the first seven peaks to their middle RT values and initially set the windows to roughly the span values. As long as they do not overlap there will be no confusion. Do not assign a reference peak. Set "find the largest peak" to ON.
2. For the rest of the compounds you can set the following:

    a. The retention times to the middle values,
    b. The absolute window to 0.12 minutes,
    c. The relative window to zero,
    d. Pentane as a reference peak, and set the largest peak in the window to ON. Set the pentane absolute window to 30 seconds.

3. For the last two compounds you should use the middle retention times, and possibly use "find the largest peak" depending on how your chromatogram looks.

There is no hard and fast rule and you should expect to *improve* your data set over time by adding to the middle RT information. This is not a static process. You must periodically improve and adjust your data set (component list). As long as you do not redefine the middle RT point accidentally in GME, you should do well. Do not forget, only use GME to *review* your data, and exit quickly without saving.

The calibration standard gas is not suitable for defining the working method used for ambient air. There are several reasons for this, but probably the most important is that the standard is at a much higher concentration than samples and usually at a different humidity from the ambient. You do not really need a method to identify most calibrations anyway, since you can tell which peak is which just by looking. There are no confusing unknown peaks (usually) which make life challenging when you are operating down at the detection limit. So do not be afraid to have a separate method for the standard if necessary. It is almost like a separate analysis. You may also consider a separate method for blanks.

VARIATIONS

Difficult target peak identifications like those discussed briefly in ***PAMSGram*** #14, are worth revisiting. The central peak in the trio is always the largest (it seems) anywhere in the USA. Since it is always present you can use it as a reference.

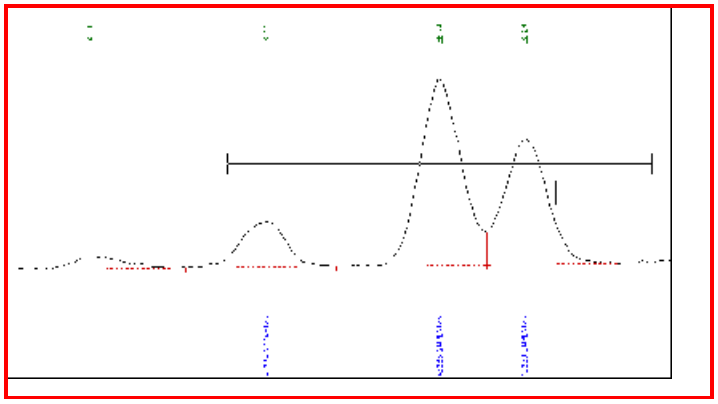


Figure 14 - The Three Amigos

The central reference peak in **Figure 14** is permitted to have a wide window and "largest peak in the window" is set ON. In this Figure, the central peak has a 14-second window, and the adjacent peaks referenced to it have 2-second windows. The first and last peaks are referenced to it, and have a much narrower window. There should be no problem identifying this grouping in all circumstances, except in a standard where the relative concentrations are not the same.

No hard and fast rule can be given for the final peaks since this set is affected by the state of your chromatograph and the ambient sample, but they usually respond well to having pentane as a retention time reference peak. Alternatively, isoprene can be used as another reference peak and hexene referenced to it. Examination of your data in the manner described in this ***PAMSgram*** will give you a good idea as to what you can do based on relative timing. A system that is running well should perform perfectly with a single reference peak.

After all of this, you can be sure that if peaks suddenly start to be misidentified there is probably an excellent reason. If you suddenly start losing peaks; find out WHY first. You may have a leak, a bad trap, bad carrier gas, a bad dryer, a very wet standard, or a bad column. It does not necessarily follow that you should discard months of retention data out before you eliminate the other possibilities first.

Your data base will become a first class diagnostic tool. If you have confidence in your retention data, you can spot a trend very quickly and head off a severe equipment failure. If you do see this happening, do not forget to switch to the PLOT Protect Mode immediately to preserve your column.

IN CONCLUSION

1. Do not rush. Let your system settle down before you try to make sense of the data.
2. Analyze your data carefully. Do not start off by defining reference peaks and windows until you know what they should be. Follow the guidelines given here.
3. Adjust your data on the basis of actual observation. Do not "assume" that the report is correct. Verify it before changing your middle RT data.
4. DO NOT use GME to reset any retention times.
5. DO use GME to examine your Expected Retention Times (in red, after clicking on the reference peak), THEN EXIT. Do not make changes and do not open another chromatogram.
6. Fine-tune your retention times on the basis of ambient air, not standards,

... and you should have a great 1999 season!