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Basic Operations Guide to the Analysis of Sulfur Using the Varian 3400 Pulsed Flame Photometric Detector (PFPD)

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ABSTRACT

This step-by-step manual is intended to provide instructions for first-time users in the use and maintenance of the Varian 3400 Gas Chromatograph (GC) with Pulsed Flame Photometric Detector (PFPD).

The instructions are specific for the analysis of sulfur mustard (HD) in diethyl phthalate (DEP) and are not intended as an exhaustive reference and therefore do not provide any insight into the theory of the PFPD or gas chromatographic principles. The instructions provide guidance for: preparing standards, generation of calibration curves, creation of sample lists and the selection of GC methods for the subsequent analysis of samples. There is also a troubleshooting section which outlines simple techniques to combat common problems that may arise whilst using the Varian 3400 GD with PFPD.

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Basic Operations Guide to the Analysis of Sulfur Using the Varian 3400 Pulsed Flame Photometric Detector (PFPD)

Executive Summary

This manual is intended to lead first-time users through the analysis of sulfur mustard (HD) in diethyl phthalate (DEP) using the Varian 3400 Pulsed Flame Photometric Detector (PFPD).

It is not intended to provide extensive information into the theories behind the PFPD or gas chromatographic principles, but should provide the reader with useful instructions on sample and standard preparation, generation of calibration curves and sample analysis. It also includes a section on troubleshooting which may be used to alleviate any commonly encountered problems.

Contents

1. Introduction

This manual is a basic operations guide to the analysis of distilled sulfur mustard (HD) in diethyl phthalate (DEP). It is not intended to provide detailed information on the theory and background behind the Pulsed Flame Photometric Detector (PFPD). For a more comprehensive guide to the PFPD, please refer to "Gas Chromatographic (GC) Analysis of Sulfur Mustard in Diethyl Phthalate (DEP)" by Paul A. Lancaster[1].

The PFPD offers enhanced detection for sulfur and phosphorous compounds as well as nitrogen and arsenic when analysed on a GC. The chemical sensitivity, total elimination of hydrocarbon interferences, lower gas consumption and ability to monitor a range of sulfur and phosphorus containing Chemical Warfare Agents (CWA) make the PFPD an ideal detector for these purposes.

This manual contains detailed instructions for preparing standards, generating calibration curves and creating and running sample lists as well as a short troubleshooting section to help solve any problems which may occur whilst using the Varian 3400 GC.

1.1 Getting Started

The software being used is the Varian Star Workstation Advanced Application Software version 6.4. The DSTO Varian 3400 PFPD is used almost exclusively for the analysis of HD in DEP. Therefore, the gases are continuously flowing and the column, injector and detector are set and held at the optimum temperatures for the analysis. However, if a power failure occurs or the instrument has been switched off for an extended period of time, the software may revert to the default settings (50^{o}C for injector, detector and column). If this happens, you will need to activate a method (see 1.7-1.9) and wait until the injector, detector and column reach the optimum temperatures before proceeding. To get started:

1) Ensure that the GC is turned on and a constant 'pop' of the PFPD can be heard[2].
2) Ensure that the auto sampler is turned on (green light).
3) Ensure that the extraction vent is directly over the detector[3].
4) Ensure that the column pressure is above 0 psi and remains constant throughout the analysis[4].
5) Ensure that the auto sampler waste tube is connected to the waste bottle and that it does not need to be emptied.

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[1] Lancaster, P.A, (1998), "Gas Chromatographic Analysis of Sulfur Mustard in Diethyl Phthalate", DSTO-TR-0703, Defence Science and Technology Organisation.

[2] If GC is turned on but it is not 'popping', ensure that all gases are available, the instrument is tuned properly (see Appendix A) and that the correct flow rates are operating (see section 4.2.2). You may then need to reactivate the method.

[3] The extraction vent is a safety measure to remove any harmful substances; it has also been shown to improve the sensitivity of the PFPD.

[4] The column pressure should be approximately 3 psi for a 30 m column and slightly less for a 15 m column when specified gas flows are used.

6) Switch on computer and log on - for username and password see lab officer in charge (OIC). Figure 1 illustrates the Star toolbar that will appear at the top of the screen after you have successfully logged in.

Figure 1: Star Toolbar

7) Click the 'System Control/Automation' button (first button from the left on Star toolbar).
8) When the Status window and ADCB window have fully loaded, click on 'File', go to 'Activate Method'.
9) Activate the most recent method file.
10) Inject an old standard or sample to ensure that the instrument is operating correctly, i.e. you can see the peak, and it has the appropriate sensitivity and retention time. For instructions on running a single sample, refer to section 3.1.1
11) If the sample runs correctly proceed to section 2. Otherwise you may need to troubleshoot the instrument (section 4).

2. Generating a Calibration Curve

2.1 Making HD Standards

Based on the sensitivity of the PFPD and the expected concentrations of the samples to be analysed, a series of standards in DEP are typically made in the range of 0–4 µg/mL (specifically 0.3, 1, 2, 3 and 4 µg/mL)[5]. The PFPD response to sulfur is quadratic; consequently, a quadratic correlation with peak area will be observed.

2.2 Creating a Calibration Method File

1) Run a standard through the GC in order to determine the correct retention time for mustard (refer to section 3.1.1). Ensure that most recent method is used.
2) Click on the 'View/Edit Methods' button (second from left in Star toolbar).
3) Click 'File' then 'Open an Existing Method'.
4) Click on 'Most Recent Method' and 'Save As'.
5) Save the file as the current date so users can easily select the most recently calibrated method when running a sample list.
6) Click on 'Peak Table', which is under the 'Data Handling' heading (see figure 2).
7) Check that the retention time is correct for HD. If it isn't, change it. Insert the standard concentrations (to three decimal places) into the 'Level Amount' columns (see figure 2, RHS).

[5] For instructions for making standard solutions, refer to Appendix B.

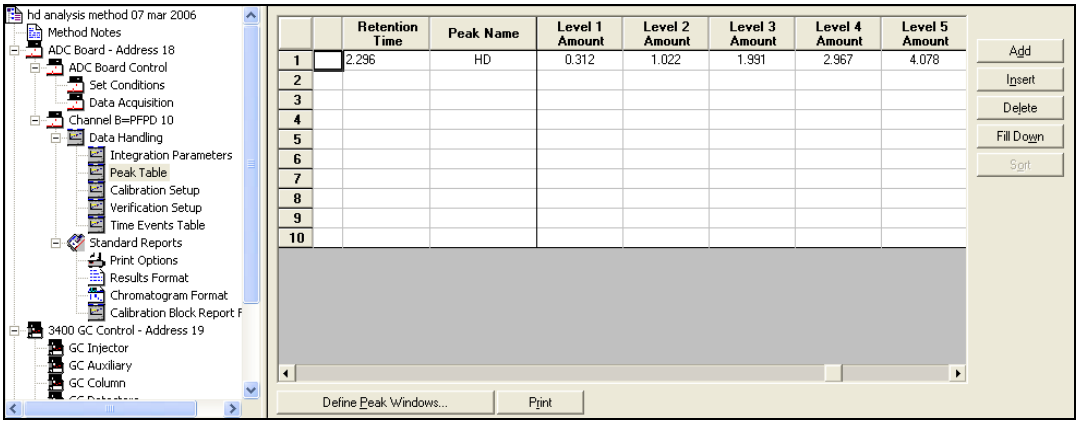


Figure 2: 'Peak Table' Window in Method Builder[6]

8) Go to 'Calibration Setup', which is also under the 'Data Handling' heading.
9) Ensure that the 'Number of Calibration Levels' agrees with the number of levels in the 'Peak Table'. If it doesn't, correct it.

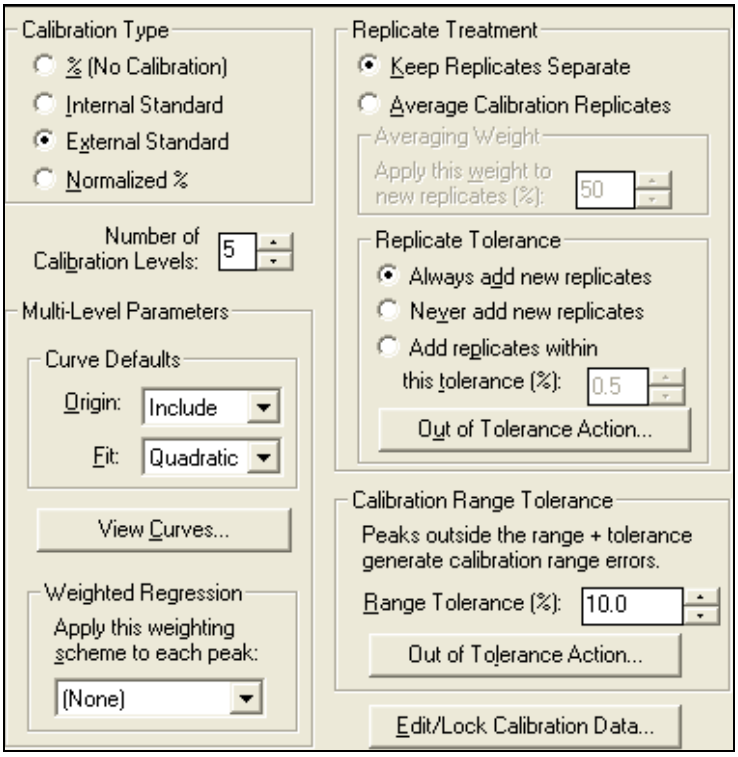


Figure 3: Calibration Set-up Screen

10) Check that Origin = Include (under 'Curve Defaults', see figure 3).

[6] The retention time in this example relates to a 30 m column. For a 15 m column the retention time is approximately 0.75 minutes; however, these may change due to gas flow rates.

11) Check that Fit = Quadratic (under 'Curve Defaults', see figure 3).
12) Click on 'Save'.
13) Close the Method Editor.
14) In 'System Control/Automation', click 'File', go to 'Activate Method'.
15) Select the method created above.
16) The method is now active and you are ready to create the calibration sample list.

2.3 Creating a Calibration Sample List

1) Place the standards in increasing concentration order in the carousel from position marked 'rack 1, vial 1' to 'rack 1, vial 5'
2) Place the blank DEP in the 'rack 1, vial 6' position.
3) Click on 'File', go to 'New Sample List'[7] in 'System Control/Automation'.
4) Name the file and click 'Save' – an empty sample list should appear on the screen.
5) In the first row under 'Sample Type', select 'New Calib. Block' from the drop down box (see figure 4)[8].

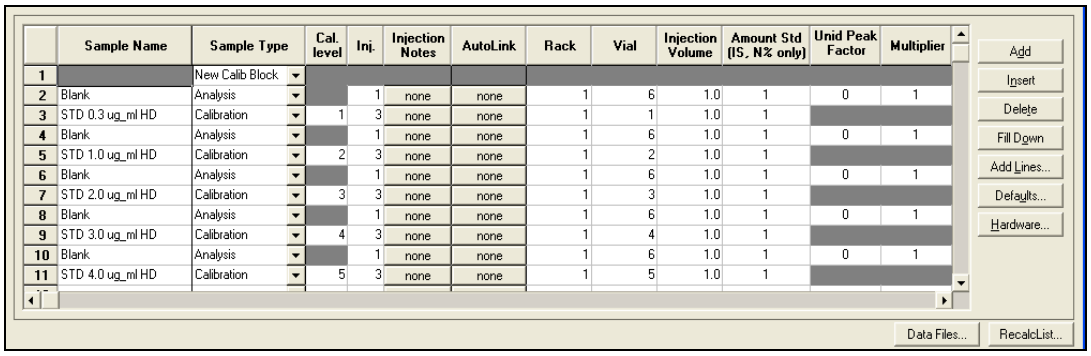


Figure 4: Example of a Calibration Sample List

6) From the second row, fill in the boxes with the appropriate data (see figure 4). For standards, under the 'Sample Type' heading select 'Calibration' from the drop down box (see figure 4)[9].
7) Blank samples should be run between standards. When using a blank sample, select 'Baseline' or 'Analysis' as the 'Sample Type'. By selecting 'Baseline' you enable the option of subtracting the baseline from future analyses.
8) The 'Cal. Level' should correspond to the 'Level Amount' number in the 'Peak Table' (figure 2).

[7] To use a pre-existing calibration sample list, go to 'Open Sample List' and click on the most recent calibration sample list and 'Save As' the current date. Check that steps 2.3.5-2.3.17 are correct. Alternatively, you can modify the existing calibration sample list.
[8] By selecting 'New Calibration Block', all previous calibration data files will be deleted from the method. If you would like to add additional data files to the method, ensure that 'New Calibration Block' is not included in the sample list.
[9] It is a good idea to run triplicate injections of the standards. This will result in three data points for each standard on the calibration curve. To run triplicate injections, insert a'3' into the 'Inj.' column.

9) Click on 'Data File', located at the bottom of the 'Sample List' window. A separate window will open as appears in figure 5.

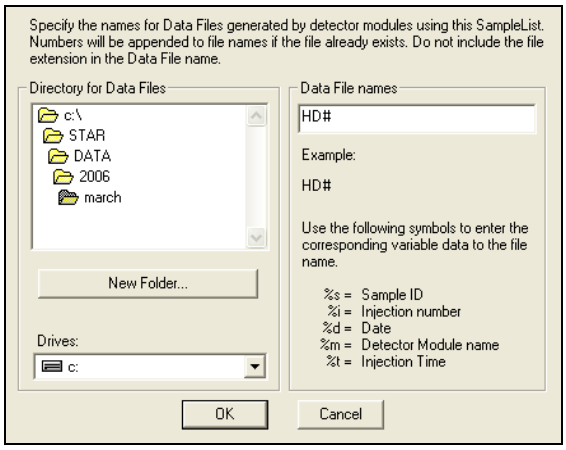


Figure 5: Data File Window

12) Ensure the data is being saved in the correct folder (current year/current month).
13) Check 'Data File names' reads 'HD#' and click 'OK'.
14) Click 'Begin'.
15) Click 'OK' in the 'Instrument Parameters' window.
16) Confirm correct method file or you may need to browse for the appropriate method as the program will automatically revert to the most recently activated method.
17) Click 'OK' to begin automation.

2.4 Viewing a Calibration Curve

Once the calibration sample list has finished running (approximately three hours), the calibration curve can be viewed to ensure it meets the calibration requirements. The calibration curve should have a regression of R^2 >0.98.

1) Click on 'View/Edit Method'.
2) Click on 'Open an Existing Method'.
3) Click on the method created (above).
4) Go to 'Calibration Setup' (see figure 3).
5) Click on 'View Curves'. The regression value can be seen in the right-hand-side top corner of the window (see left-hand side of figure 6)[10].
6) The mouse can be used to expand certain regions. To revert to zoomed out curve, double click on 'Small chromatogram' (top left of enlarge region, right-hand side of figure 6).
7) Outliers can also be excluded from the curve by right clicking on the specific data point or by double clicking on the specified data point. Double clicking opens the

[10] The curve can also be viewed from the 'Interactive Graphics' program (third icon from the left on the Star toolbar)

'Point Info' box. Tick the 'Exclude Selected Point From Calibration' box to exclude the data point (see figure 7).

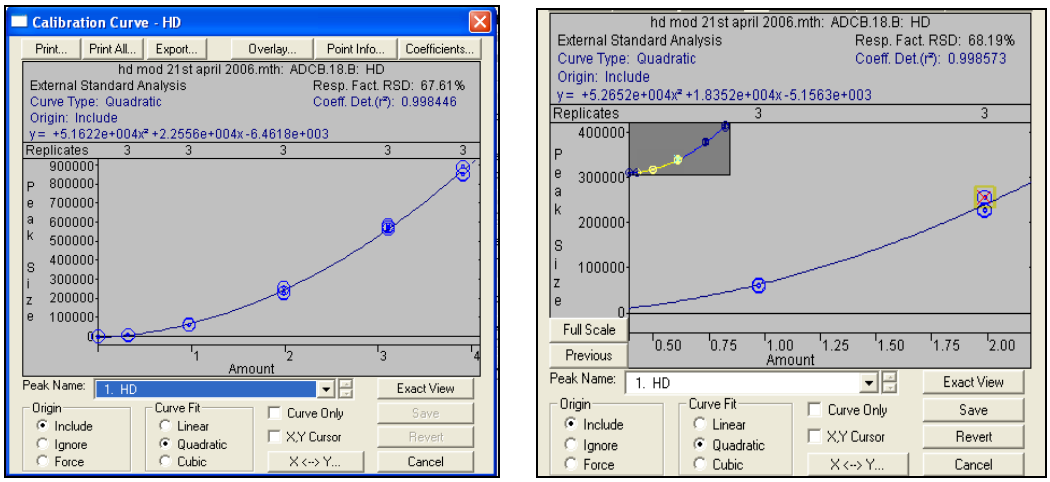


Figure 6: Calibration Curve (left) with Expanded Region (right)

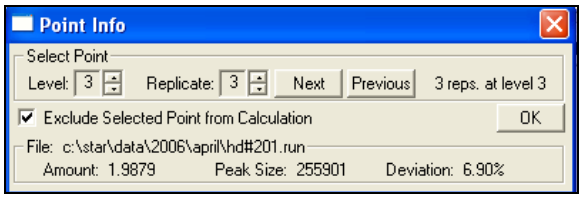


Figure 7: 'Point Info' Box

3. Analysis of Samples

3.1 Running Samples

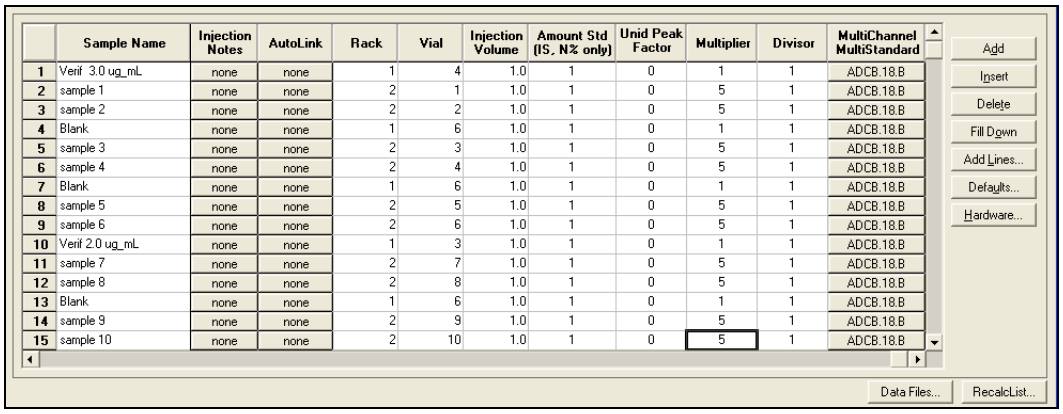
Place sample vials for analysis in the carousel, usually from rack two onwards.

3.1.1 Running a single sample

1) In the 'System Control/Automation' window click 'Inject'.
2) Go to 'Inject Single Sample'.
3) Insert the sample name and the correct rack and vial number of the desired sample.
4) Check that the most recent method is used. If not, browse for the correct method.
5) Click on 'Data File'.
6) Open the appropriate folder (year/month).
7) Change 'Data File Names' to HD#, click 'OK'.
8) Click the 'Inject' button.
9) Automation will begin.

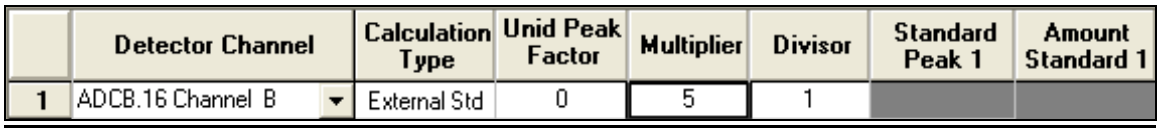
3.1.2 Running a sample list

1) In 'System Control/ Automation' window, click 'File'.
2) Click 'New Sample List'.
3) Name the new list, click 'Save'. A blank sample list should appear[11].
4) Insert the details of each sample.
5) Verification and blank samples should be run throughout the sample list for quality control checks (see figure 8). The 'Sample Type' of a verification standard should be set to 'Verification'.
6) Diluted samples require a multiplier to be added. This is added in both the 'Multiplier' column and in the 'Multichannel' column[12].
7) Click on the multichannel 'ADCB' button in each row and insert the correct value into 'Multiplier' (see figure 9).
8) Click on 'Data file' (see figure 8).
9) Open the appropriate folder (year/month).
10) Change 'Data File Names' to HD#, click 'OK'.
11) Click 'Begin'.
12) Click 'OK' in the 'Instrument Parameters' window.
13) Confirm correct method or browse and select desired method.
14) Click 'OK' to begin analysis.
15) Automation will begin.



	Sample Name	Injection Notes	AutoLink	Rack	Vial	Injection Volume	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChannel MultiStandard
1	Verif 3.0 ug_mL	none	none	1	4	1.0	1	0	1	1	ADCB.18.B
2	sample 1	none	none	2	1	1.0	1	0	5	1	ADCB.18.B
3	sample 2	none	none	2	2	1.0	1	0	5	1	ADCB.18.B
4	Blank	none	none	1	6	1.0	1	0	1	1	ADCB.18.B
5	sample 3	none	none	2	3	1.0	1	0	5	1	ADCB.18.B
6	sample 4	none	none	2	4	1.0	1	0	5	1	ADCB.18.B
7	Blank	none	none	1	6	1.0	1	0	1	1	ADCB.18.B
8	sample 5	none	none	2	5	1.0	1	0	5	1	ADCB.18.B
9	sample 6	none	none	2	6	1.0	1	0	5	1	ADCB.18.B
10	Verif 2.0 ug_mL	none	none	1	3	1.0	1	0	1	1	ADCB.18.B
11	sample 7	none	none	2	7	1.0	1	0	5	1	ADCB.18.B
12	sample 8	none	none	2	8	1.0	1	0	5	1	ADCB.18.B
13	Blank	none	none	1	6	1.0	1	0	1	1	ADCB.18.B
14	sample 9	none	none	2	9	1.0	1	0	5	1	ADCB.18.B
15	sample 10	none	none	2	10	1.0	1	0	5	1	ADCB.18.B

Figure 8: Example of a Sample List for Analysis



	Detector Channel	Calculation Type	Unid Peak Factor	Multiplier	Divisor	Standard Peak 1	Amount Standard 1
1	ADCB.16 Channel B	External Std	0	5	1		

Figure 9: Example of the Multichannel Window for a Sample Diluted 1 in 5 Times

[11] A previous sample list may be copied and pasted into the new sample list or you can 'Save As' a new sample list. Generic sample lists have also been created that can be used, such as neat samples, 1 in 5 dilution and 1 in 10 dilution.

[12] If the multiplier is not added into the multichannel column, it will not take effect. This is a software glitch.

3.2 Creating a Sequence List

If you want to run one sample list directly after another sample list you can create a 'sequence list'. This is useful when you would like to generate a new calibration curve followed by samples using that new calibration curve file. Sequence lists also allow you to use different methods for the same samples[13].

1) Click 'File'.
2) Go to 'New Sequence List', name it and click 'Save' (see figure 10).
3) Select 'Inject' from the 'Action' box.
4) Browse for 'Method' file and for 'Sample/Recalclist'[14]
5) Repeat this process for each sample list that you want to run.
6) Click 'Begin'.
7) Click 'OK' in the 'Instrument Parameters' box.
8) Click 'OK' (System Control).
9) Automation will begin.

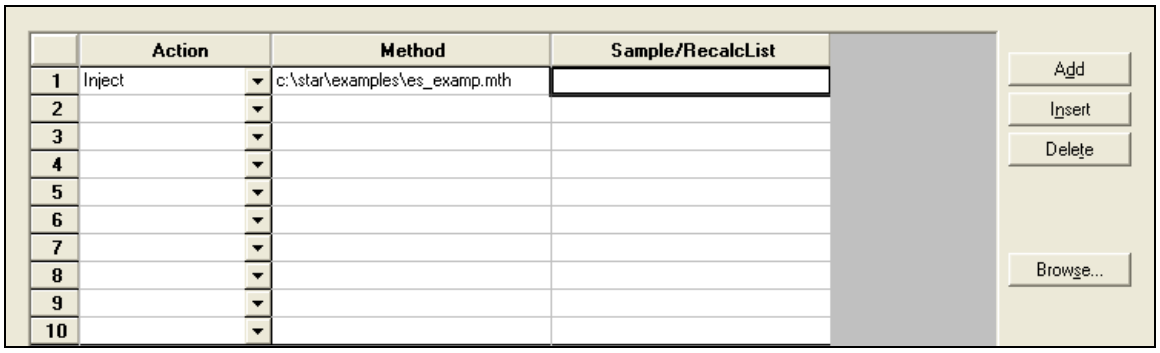


Figure 10: Example for Creating a Sequence List

3.3 Viewing Chromatograms

1) Click button third from the left on the Star toolbar ('View/Edit Chromatograms')
2) Click 'File'.
3) Click 'Add/Remove Chromatogram'
4) Find desired chromatogram and click 'Add to List' or double click on 'File Name'. You can view up to seven spectra at once (see figure 11)

[13] You cannot create a sequence list (or a new sample list) if an automation is in process. You must first suspend the automation and resume after the new list has been created.
[14] Ensure the sample list will be saved in the correct data file and with the correct data file name (refer to steps 3.1.2.8 and 3.1.2.9 above).

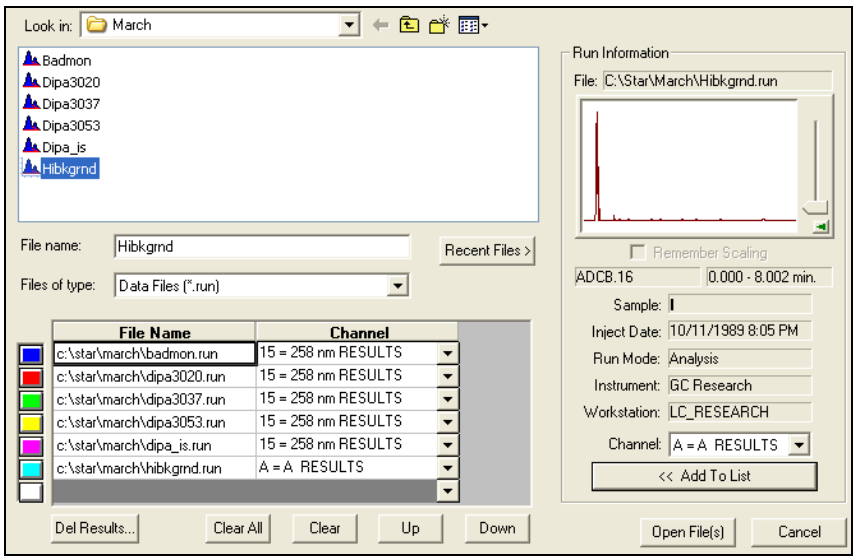


Figure 11: 'View Chromatograms' Window

5) Click 'Open files'.
6) A blue screen revealing chromatograms should appear (see figure 12).
7) Use your mouse to expand region of interest.
8) To highlight a specific chromatogram, click on the appropriate colour box above the chromatograms; blue, red, green, etc.
9) HD peak should be identified and labelled.
10) To view information about the sample right click on the spectrum of interest (see figure 12). The coloured bar at the bottom of the list (pink) indicates that it is showing information about the pink chromatogram.

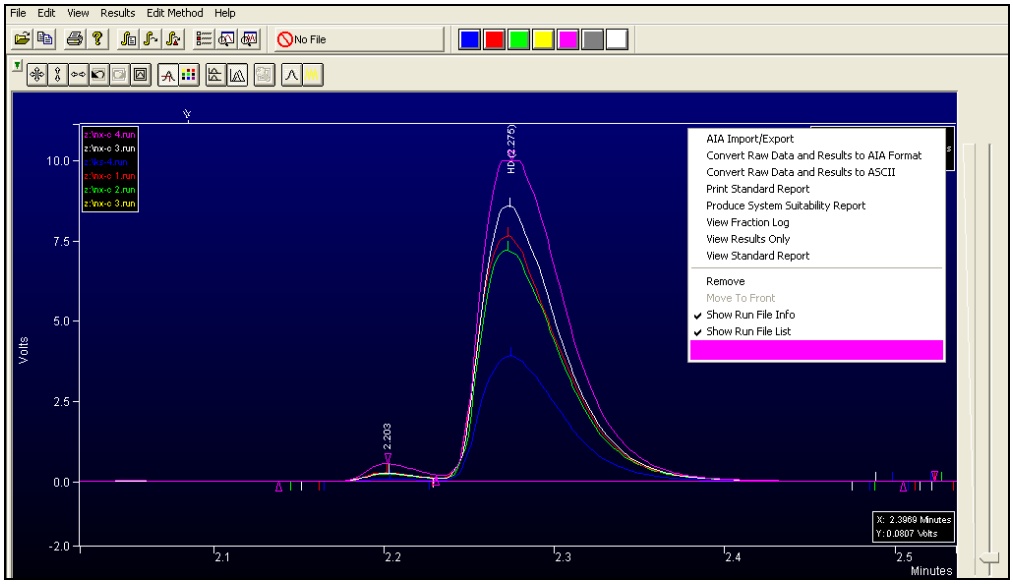


Figure 12: Chromatogram Window

11) Go to 'View Results Only', which will provide the following information (see figure 13):

i. The method file
ii. The sample ID
iii. Injection date
iv. The concentration of HD (under 'Result' heading) [15].
v. Retention time
vi. Peak area
vii. Multiplier number

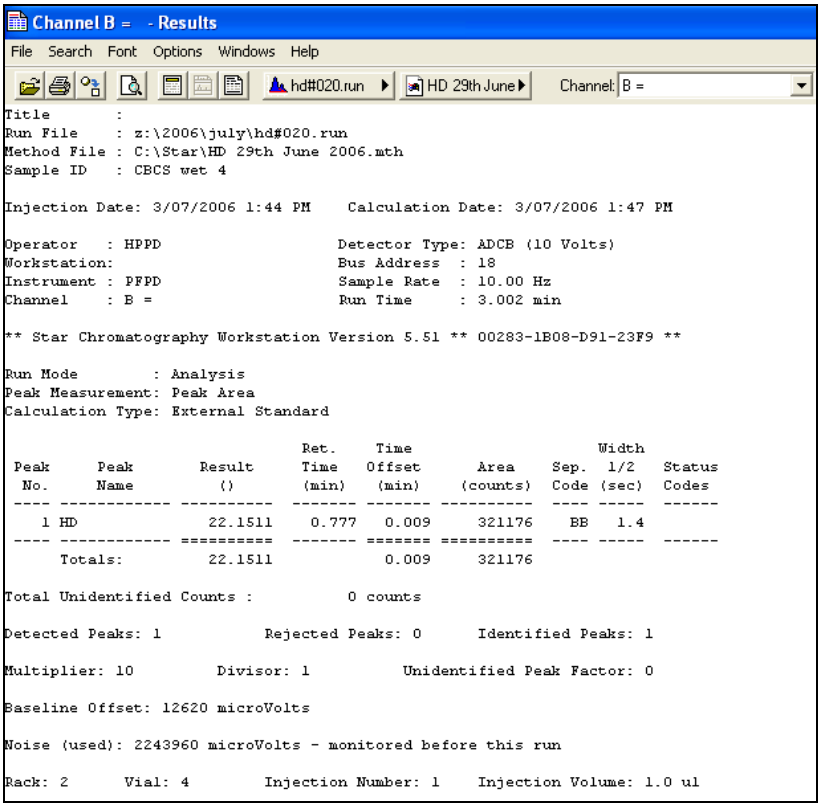


Figure 13: 'Results Only' Screen

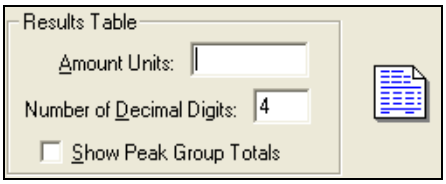


Figure 14: 'Results Table'

[15] The units of concentration can be set in the method. Go to the 'Results Format' heading and insert the appropriate units in the 'Amount Units' box (see figure 14).

4. Troubleshooting

4.1 Automation Stops Prematurely

If the automation stops halfway though the sample list, a fault may have occurred. You may be able to finish the list by deleting the samples that have already run and restart the automation from where it stopped. However, if the fault is anything other than a software glitch, the automation is likely to stop again. In order to identify the fault that has occurred, check the instrument log first to see if this has identified the cause. Otherwise, an instrument check must be performed.

4.1.1 Instrument check

On the GC unit press the 'Instr. Test' button. The 'Instr. Test' is a second function button; therefore, press 'Shift' and 'Instr. Test' simultaneously. This process will start a series of checks of the instrument. At the end of the check, the GC will display a message that states which faults, if any, have occurred as a fault number or it will display a 'Test OK' message.

4.1.1.1 Fault number

To scroll through the faults, press 'Enter' at each fault number. After the last fault number is displayed, the 'Return to Test Menu?' message will appear. Press 'Yes'. The GC will then give you the option to perform GC tests (refer to 4.1.2). Details of the fault numbers can be found in either the Varian manual or the auto sampler manual. Both manuals will contain details on how the problem may have occurred and how to fix the problem.

4.1.1.2 Test OK

If the instrument check displayed the 'Test OK' message, press 'Enter'. This will display the 'Return to Test Menu?' message. Press 'Yes'. The GC will then give you the option to perform GC tests (refer to 4.1.2).

4.1.2 Performing GC tests

The test options are calibrate plunger, vent test, relay test, key echo test and destructive RAM test[16].

To by-pass any of the tests, press 'Enter' at each of the tests.

To perform any of the tests, press 'Yes'.

[16] A destructive RAM test often clears most electronic-based glitches. To perform a destructive RAM test, press 'Yes' when this option is offered. Give the GC a few seconds and switch it off and back on again. In order to start the flame popping, you will need to reactivate the method (see 1.1.8). You may first need to restart the computer program.

When all test options have been displayed, a 'Test Session Complete' message will be displayed. Press the 'Status' button to return to normal GC usage. Once the fault has been rectified, the sample list can be restarted from a specific line.

4.2 Peak Retention Time Shift

Any problems that occur with gas flows can have an effect on the peak retention time. The most common gas flow problem is an old or leaky septum, which will result in a gradual increase in retention time as automations proceed. A leaky septum will cause the column pressure to decrease.

4.2.1 Changing the septum

1) Switch the auto sampler off.
2) Open door on auto sampler.
3) Release latch and open carousel module.
4) Unscrew the injector port. Take care as this will be hot ~ 270 ^{o}C.
5) Replace old septum with new septum using tweezers.
6) Screw injector port back in place (not too tightly).
7) Switch the auto sampler power back on with the carousel door still open. This will allow you to raise the injector and lower it to puncture the septum. Puncture the septum manually three to five times. This will minimise needle damage during automation.
8) Raise the injector fully and lock back into place.
9) Close the carousel door and the auto sampler should do a quick self test before it is ready.

Column pressure should rise slowly. The pressure gauge should show a column pressure of 1-3 psi. This value is dependent on the length of the column and carrier gas flow rate. If you find that the gauge does not change after the septum was changed, then there may be a leak elsewhere in the system. Check to see that all gases are available and that the connections to the GC are tight. If this is not the problem, switch the GC off and wait for the fan to stop turning. Open the oven door and use a spanner to ensure that the capillary column nuts are tight. Keep in mind that surfaces will be hot. If the column pressure does not rise, make sure that there are no anomalies in the column such as a break. If this is not the problem, the gas flow rates may need to be checked and reset.

The flow rates which will optimise the PFPD for sulfur detection in DEP are:

Column flow = ~ 2 mL/min
H_2 = 11.87 mL/min
Air 1 = 14.76 mL/min
Air 2 = 10.02 mL/min

4.2.2 Checking flow rates

1) Switch GC off.
2) Attach flow meter to screw thread on detector.
3) Note the values at which each of the dials is set.
4) Turn H_2, Air 1 and Air 2 dials anticlockwise so that they won't turn any further. Use the flow meter to determine the column flow speed. The column flow dial is located inside the GC cover above the pressure gauge.
5) Adjust the flow dial so that the flow speed is approximately 2 mL/min.
6) Adjust the H_2, Air 1 and Air 2 in the same manner so the flow rates are close to the values stated above[17].
7) When finished, switch the GC back on.
8) The PFPD should 'pop' twice every second, although up to four times is still considered normal.
9) The GC will then need to be tuned (see Appendix A).
10) Run a single sample in order to determine the retention time and to be certain that the solvent front comes through within the run time[18].
11) If chromatogram looks good, a new calibration curve can then be run[19].

4.3 PFPD Pulsing Speed

4.3.1 Flame pulsing too fast/too slow

It is acceptable for the flame to pulse up to four times per second. However, the normal pulse rate is around two pulses per second. If it is pulsing too fast or too slow, it is most likely a problem with the gas flow rates and/or tuning. For flow rates see 4.2.2 and for tuning see Appendix A.

4.3.2 Flame does not ignite

Reactivate the method: If the flame is not pulsing then you may need to reactivate the method.
Detector coil broken: This can be checked by using a mirror to check whether the igniter (back of detector) is glowing.
Leaking gas: Use a soapy solution to check that there is no gas leaking from around the washers and the seals.
Gas flows: Check that gas is available and that the gas flows are not too low.

[17] This can be done cumulatively. For example if the column pressure is 2 mL/min, the flow that you want for H_2 is 13.87 mL/min (2 mL/min + 11.87 mL/min) and the flow rate for Air 1 would be 28.63 mL/min (13.87 +14.76) etc.
[18] When the DEP solvent is being detected, the PFPD will usually slow down or even stop. Be certain the popping is resumed before the end of the run time; otherwise the solvent will remain in the column for the next run.
[19] A new calibration curve is required each time the flows are changed or the GC is retuned.

Gas composition: Check if the igniter is glowing dimly. If so, the gas composition may be too rich in hydrogen. Check the air flows (air 1 and 2) and adjust the hydrogen flow until the flame pops.

4.4 Elevated Baseline

Dirty Combustor: Replace the combustor with a clean one[20].
Dirty Column: Replace the dirty column with a new one. Alternatively, you can cut the first 0.5 m from the injector end of the column or bake the column (see notes supplied with column).

4.5 No Peaks

Column: The column may be broken or extends too far into the combustor. Reinstall the column (see the manual for correct distance to insert column into injector and detector).
Auto injector: Check for leaks or a blocked syringe.
Photomultiplier Tube (PMT): The Photomultiplier Tube may be broken. This will need to be replaced.

[20] Refer to "Varian Chromatography Systems, Pulsed Flame Photometric Detector", Operators Manual, p. 99, "Replacing a Dirty Combustor".

Appendix A: Tuning the PFPD

If any of the gas flow rates have been adjusted, it will be necessary to tune/optimise the GC. This is done by altering the air/hydrogen split ratio.

1) Click the 'System Control/Automation' button and wait until you can see the GC trace.
2) Turn the 'Air/Hydrogen Ratio' knob clockwise until you can't turn it anymore[21].
3) Slowly turn the 'Air/Hydrogen Ratio' knob anti-clockwise until the trace suddenly shoots up.
4) Trace should shoot up a second time before exponential decay.
5) Turn slowly until the trace begins to decrease in an exponential manner. This may take anywhere from 4 to 7.5 turns.
6) Stop turning when trace is approximately at half the height of the second peak ('Normal Operation', see figure 9).
7) Note how many turns required and record in the PFPD diary.

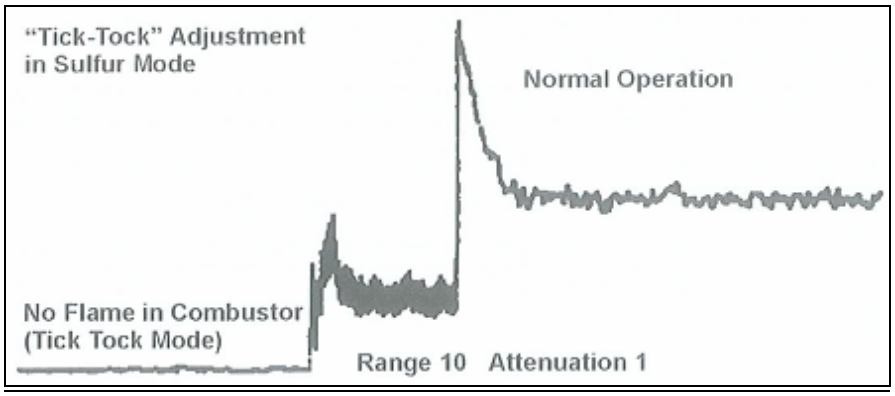


Figure 15: Optimisation of GC Trace

[21] The Air/Hydrogen ratio knob is located on the front left side of the GC, directly above the hydrogen dial.

Appendix B: HD Standard Preparation Notes

The following is a calculation for producing a 0.3 μg/mL standard solution. In order to do this, we make a stock solution from that we will further dilute. This is done to minimise the risks associated with handling neat/concentrated agents.

Pure HD is drawn into a 50 μL graduated syringe that dispenses in 1 μL increments. The desired stock solution concentration is ≈ 300 μg/mL of HD. Weigh out approximately 30 mg (around 20–25 μL) of HD into a 100mL volumetric flask and make up the volume with DEP.

Actual mass of HD weighed = 29.7 mg
Concentration of Stock 1 = 297 μg/mL

A Stock 2 solution of ≈ 50 μg/mL (from stock 1) in 25 mL volumetric flask is required.

To work out an approximate mass required, use equation $C_1V_1 = C_2V_2$ where:
C_1= 297 μg/mL
V_1 = X
C_2 = 50 μg/mL
V_2 = 25 mL

Therefore, X = (50μg/mL x 25mL)/297μg/mL
Therefore, X = 4.208 mL

However, we want the value in grams, so we have to use the density of DEP, which is 1.115 g/mL. Therefore, approximate mass required: 4.208 mL x 1.115 g/mL = 4.692 g.

Actual mass weighed to make accurate concentration= 4.500 g
Therefore, actual volume for accurate concentration = (4.500g)/ (1.115g/mL) = 4.035 mL
Thus, actual accurate concentration (C_2) = (297 μg/mL x 4.035 mL)/ 25 mL

Concentration of Stock 2 = 47.946 μg/mL

Standard 1: 0.3 μg/ mL in a 25 mL volumetric flask. Therefore:
C_1= 0.3 μg/mL
V_1 = 25 mL
C_2 = 47.946 μg/mL
V_2 = X

Therefore, X = (0.3μg/mL x 25 mL)/ 47.946 μg/mL
X = 0.156 mL or 0.174 g
Actual mass weighed = 0.180g or 0.161mL
Therefore, Standard 1 = 0.308 μg/mL

This same process is used to make the 5 standard solutions.

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Rachel Moore

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19. ABSTRACT

This step-by-step manual is intended to provide instructions for first-time users in the use and maintenance of the Varian 3400 Gas Chromatograph (GC) with Pulsed Flame Photometric Detector (PFPD). The instructions are specific for the analysis of sulfur mustard (HD) in diethyl phthalate (DEP) and are not intended as an exhaustive reference and therefore do not provide any insight into the theory of the PFPD or gas chromatographic principles. The instructions provide guidance for: preparing standards; generation of calibration curves; creation of sample lists; and the selection of GC methods for the subsequent analysis of samples. There is also a troubleshooting section which outlines simple techniques to combat common problems which may arise whilst using the Varian 3400 GD with PFPD.