

STEP BY STEP METHODS FILE

1. Start by telling the computer which instruments you are using: the autosampler, the oven, and the detector.

On the desk top click on: **Instrument Setup Icon**

The screen opens with available devices listed in the left hand side. Click to move to right hand side, then click the lower right button “configure”

AS2000 Autosampler (autosampler)

Serial port = through GC

Autosampler = left

Number of vials = 90

maximum syringe volume = 10 uL

Trace GC 2000 (gas chromatograph)

Tab General

Connection

Serial port = through GC

Xcaliber is programmed so that it self tests the equipment it is working with. If you are on site and creating a methods file the instrument would test to see that it's serial port connection is “through GC”. Building a methods file off the instrument means that certain parameters will have to be adjusted when you go on line. Off line your choices for serial ports are com1, com2... You will need to change this once you upload your methods file to be serial port ‘ through GC.’

Net work address = not active

Option

Pressure units: psi

Tab: Inlets

Left = SSL

Right = PTV

Right Valve button active: check “solvent valve”

Tab: Detectors and Data

Detectors: all should display “none”

Option: only active box should be 60Hz

Data Channel Definitions: All Channel numbers should display “none”

Channel Names: should display “Channel 1; Channel 2; Channel 3”

No other buttons should be activated.

Tab: Auxiliary and Oven Options

No buttons should be highlighted.

Exit by hitting “OK”

Trace MS Plus (mass spec detector)

When the devices are selected, hit “done”

2. From Desktop, click on “Xcaliber”: Top bar tab: Click on “on-line” tab.
3. At upper left click on “file” and create a “new” method file name

Method 1: Finding the Peaks

1. click on Left images - **AS2000 Autosampler**

A. K Tab: Injection Events

Sample Cleans -

refers to the number of times the syringe is rinsed with the sample, the volume of the rinse is specified by “pull-up: volume”. A typical number of rinses with the sample is 3. **We use 2.**

Pull-Ups

- a. **Number of pull-ups** - is the number of times the syringe plunger is pumped to eliminate air bubbles when we pull the sample or solvent or wash into the syringe. **We use 3.**
- d. **volume** This is the volume of sample used to rinse the syringe. This volume is thrown away. **We used a value of 5 uL.** (Typical value is 5 uL.)
- e. **Delay** This is the number of seconds between each pull up (rinse). If the sample is viscous then you want a longer time between each pull up. **We use 1s.**

Injector Control

- a. **Sample volume** (range 1-5 uL). **We use 1 uL.**
- b. **Air volume** is used to create gaps between samples: typical volume is 1 uL. **We use 1 uL.**
- c. **Injection delay** is the time the needle remains in the injector after the plunger has been depressed: - suggested value is 3-4 s. **We use 4 s.** This time is delayed in order to allow the needle to reach the temperature of the injection port. If the temperature of the needle is colder than the temperature of the injection port the sample will rapidly expand and “blow out” of the injection port. If the temperature of the needle is hotter than the injection port the sample will condense within the injection port. Either way the delivery of the sample to the column is poor and not reproducible.
- d. **Pull-out delay** is the time the needle remains in the injector after the plunger has been depressed. Suggested value is 0 s. **We use 0 s.**
- e. **Injection speed** 50 uL/s
- f. **Pull-up Speed** 10 uL/s

B. Autosampler K tab: Pre/post events

Pre-injection Washes

- a. Number of rinses - typical value 3 (**We use 3**).
- c. Volume (uL) - typical value 3 (**We use 5**).
- d. Pre-injection Solvent - typically we use Solvent "A" as the wash solvent.

Post-injection Washes The purpose of this would be to clean the syringe at the end of the injection - if, for example, this is the last sample.

- a. Number of rinses - 3
- b. Volume 5
- c. Post - injection Solvent - this could be different from "A" if you need to clean the syringe from material that adheres to syringe interior.

2. **Trace GC 2000 (Oven Set up)**: (Click on left hand side)

A. TAB: OVEN

The oven conditions can be optimized by the student, or can be optimized to match the data for the Supelco Standards shown in "Chromatography Module". We will match the Supelco operating conditions.

Initial: Temp: 100 °C; Hold Time 2.00 minutes

Ramp 1: Rate: 12C/min; Temp: 280 °C; Hold Time 3.00

Post Run Conditions - leave unused

Oven Max Temp 350 °C; Prep Run Timeout (10 min.); Equilibration time (0.5 min.)

B. Tab: Left SSL

Mode: Split

No other boxes activated

C. Tab: Left Carrier

No boxes activated; options: constant pressure

D. Right PTV

Mode: PTV Splitless

Inlet

activate Temperature 35 °C

Split Flow 50 ml/min

Splitless Time 1.00 min

Solvent Valve Temp. 100 °C (check box)

Purge

check: Constant septum purge

no other boxes activated

Surge

no boxes activated

Injection Phases

Injection: Time 0.05 min (no other boxes activated)
Evap: no boxes highlighted
Transfer: Pressure (not activated); Rate: 14.5 °C/min; Temp 260 °C; Time 1.00 min
Cleaning: nothing activated

Options

Nothing activated

E. Right Carrier

Flow: on; 1.0 mL/min
Options: Constant Flow
Check: vacuum compensation
Gas Saver - not checked

G. Run Table

not used.

3. TRACE MS PLUS

Maximize the screen to be able to better see all the options

For our initial method to find our peaks choose:

Add Full

Mass Range: 45.00-400.00

Time: 6.00-20.00 min

Peak Format: Centroid - allows the scan to occur more rapidly (saves time)

Scan time 0.20 min

Multiplier 250 V

Unhide per method parameters: Ionization mode EI+ (70 eV)

Source Temperature: 200 °C

Interface Temperature: 275 °C

You should be able to obtain something that looks like Figure 1 when you run this method for the Supelco Standards on Loyola's GCMS. The peak retention time (rt) in minutes, the width of the peak (in seconds), and mass peaks associated with each chromatographic peak and the assignment of the chromatographic peaks based on the mass spectra are shown in the table below.

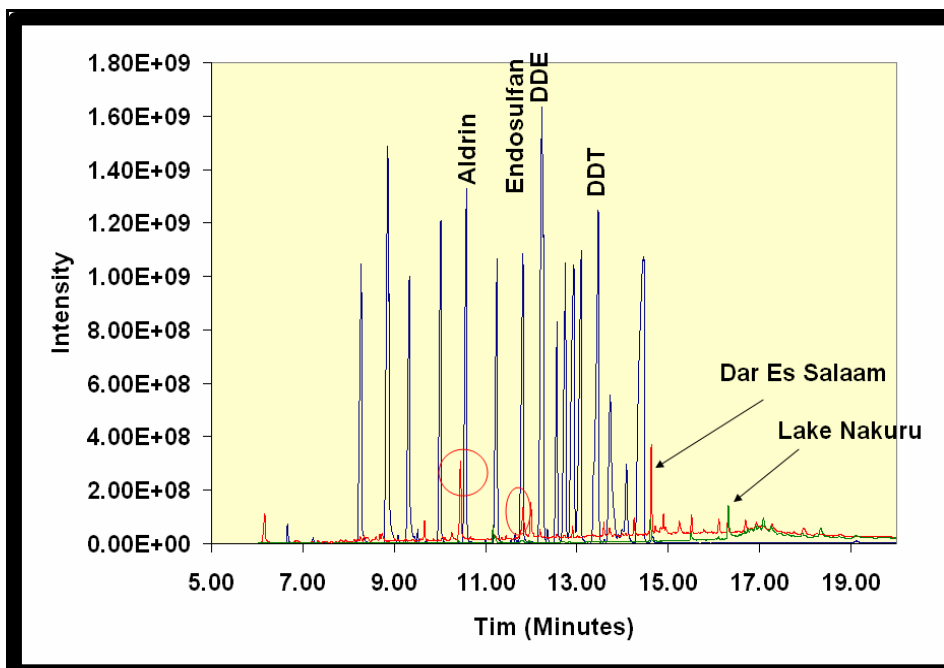


Figure 1 Total ion chromatograph of Supelco Pesticide Standards and several scans associated with Aqueous sample from Dar Es Salaam, Lake Nakuru, and Meru.

Table 1: Data associated with Figure 1. The most intense peaks are highlighted in Yellow.

Peak Shape	Center Min	Peak Min	End Peak Min	Peak S	Assigned Identity	Mass Spectral Peaks; Largest One is highlighted										
	8.29	8.2	8.3	6	Unknown	100	102	135	235	237	270	272	274	276	278	337
sh	8.79	8.8	8.87	4.2	BHC	51	83	85	109	111	145	181	183	185	217	219
	8.89	8.88	8.91	1.8	BHC	51	83	85	109	111	145	181	183	185	217	219
	9.33	9.25	9.36	6.6	BHC		83	85	109	111	145	181	183	185	217	219
	10.02	9.95	10.04	5.4	Heptachlor	65	100	102	270	272	274	276				
	10.58	10.5	10.6	6	Aldrin	66	79	91	103	186	220	255	261	263	265	293
	11.25	11.15	11.3	9	Heptachlor epoxide	81	353	355	357							
	11.82	11.75	11.85	6	Endosulfan 1?	75	102	119	121	159	160?	170?	172?	193	195	197
	12.23	12.15	12.25	6	Endrin	79	81	83	105	123	140	176	210	246	248	263
sh	12.26	12.25	12.27	1.2	DDE	104	123	174	176	246	248	316	318	320		
	12.56	12.5	12.58	4.8	dieldrin	67	79	81	82	113	147	149	175?	183?	193	209
	12.75	12.68	12.76	4.8	Endosulfan II?	63	88.8	101.9	159	160?	170?	193	195	207	235	237
	12.93	12.82	12.96	8.4	DDD	165	235	237								
	13.09	13.01	13.1	5.4	Endrin Aldehyde	67	248	250	253	279	281	343	345	347		
	13.47	13.35	13.5	9	DDT	165	199	235	237							
small	13.73	13.65	13.8	9		227,26										
v. sm	14.09	14.05	14.15	6		67	315	317	319							
sh	14.45	14.3	14.4	6		227										
	14.48	14.45	15	33	Methoxychlor	114	227									

METHOD 2: Single Ion Counting

Open up Method 1 and resave with a new name: You will be altering only the MS portion of the file from a total ion chromatogram to a single ion chromatogram.

The chromatograms obtained with the Method 1 above show the standards identified as per the table above. In order to obtain better peak heights for our unknown samples we need to look at single ions within the time frame that we expect the peak to elute. For example we expect three forms of BHC to elute at 8.28; 8.86, and 9.33 minutes. The high intensity peaks are highlighted (Table 1). The masses expected for the three BHC are 109; 111; 181; 183; 217; 219; and 221. We should monitor only these peaks at those times to optimize the method for detection of these compounds

1. Click on the Xcaliber icon on the desk top
2. Click on the Instrument Setup
3. Click on the file at the top left hand side and open your old file. Save with new name.
4. Click on the left hand side icon for the Trace MS Plus.
5. At upper right hand side is the area described as “Per Method parameters”
6. Click on the “Ionization Mode” and set that to EI+
7. Move down one line and click on the Source Temperature and set that to be consistent with the ion source in the Mass Spect (200 °C).
8. Move down another line and set the interface temperature to be consistent with the GC method (250 °C).
9. If the Method editor, upper left hand side, has a set of written material followed by a grey bar with an upward arrow “Hide Method Options”; click on that grey bar
10. Click on (under Scans) add SIM
11. This opens up a box at the bottom which, in turn, allows you to highlight “mass”, “span”, “time”, etc.

Add, then highlight the line to set the parameters:

Name	Mass	Span	Time Range (min)	Dwell Time (s)	Multiplier (V)
	100	1	8.2-8.3	1	200
	181	1	8.79-9.36	1	200
	100	1	9.36-9.95	1	200
	66	1	10.5-10.6	1	200
	81	1	11.15-11.30	1	200
	195	1	11.75-11.85	1	200
	79	1	12.15-12.25	1	200
	246	1	12.25-12.27	1	200
	81	1	12.5-12.58	1	200
	195	1	12.68-12.76	1	200
	235	1	12.82-112.96	1	200
	67	1	13.01-13.1	1	200
	235	1	13.35-13.5	1	200
	227	1	14.45-15.00	1	200

In the Table “**SPAN**” refers to the range of masses that should be measured centered around the specified mass. This sets the mass spectra width. The **time range** refers to the time you expect the chromatographic peak to exit into the mass spectrometer. Time is given in 100th minutes (not seconds). The **dwel time** is related to the time we sit at one mass to count. The **multiplier voltage** refers to amplification of the signal.

You should try and set the time around which you will monitor the masses so that it sits near the center of the chromatographic peak. This can be determined by looking at the base width of the chromatographic peak obtained during the qualitative chromatogram.

Sequence File

In your sequence file you will specify to the computer which tuning file to look at; which methods to use; and which samples to apply the method to that reside within the autosampler, and to which raw data file you wish to deposit the information obtained during the run.

Click on the main image on the screen which says Sequencing. In the line indicate that you are running an unknown, where you want the raw data file to be deposited, and where the methods file is that you created. Also indicate what position each sample you are running has within the autosampler. We had specified 5 uL sample injections so write this in here. This number will overwrite whatever you specified in the instrument methods file!!!
