

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
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VOLATILES ANALYSIS by GCMS HEADSPACE ANALYSIS

PRINCIPLE

This method confirms the identity of volatile compounds in biological specimens such as blood, serum, vitreous humor, urine, tissue homogenates and gastric content. Head space techniques are based on Henry's law of physics which states that the ratio of a dissolved substance in an aqueous solution is dependent on temperature, pressure, and concentration in the aqueous medium. Volatile substances may be measured by analyzing the vapor (head space) which is in equilibrium with a liquid specimen. Biological fluids or tissue homogenates are placed into a vial with an appropriate internal standard, sealed with a septum and allowed to stand until equilibrium is reached. A sample of the head space is then removed from the sealed vial using an autosampler (or manually by syringe) and injected into a head space gas chromatograph (GCMS). Volatile analytes are separated by the column and detected and identified by GCMS. Head space gas chromatography employed in this laboratory uses ammonium sulfate as a salting-out agent to facilitate the liberation of volatiles from the matrix. The salting-out agent is added to the internal standard solution. The diluent solution also contains sodium dithionite to liberate the oxygen from the red blood cells.

SAFETY

The handling of all samples, reagents, and equipment is performed within the established laboratory safety guidelines detailed in the safety manual.

SAMPLE PREPARATION

Sample preparation is identical to that used for miscellaneous volatiles by headspace. Refer to that SOP for sample preparation.

INSTRUMENTATION

GCMS	Agilent 5973MSD with 6890N Network GC
Autosampler	Agilent 7694 Headspace Sampler or 7683A autosampler
Gas Chromatograph	Agilent 6890N Gas Chromatograph/Agilent 5973N Mass Spectrometer (GCMS)
Columns	RTX-BAC1 30 meters, 0.32 mm id, 1.8 micron film thickness
Integrator	Computer with HP-GC Chemstation software
Head space methods	MS#DVOLTP38.M

TOP LEVEL PARAMETERS

Method Information For: C:\MSDCHEM\1\METHODS\MS8DVOLTP38.M

Method Sections To Run:

- Save Copy of Method With Data
- Pre-Run Cmd/Macro =
- Data Acquisition
- Data Analysis
- Post-Run Cmd/Macro =

Method Comments:

Volatile (ethanol) method by headspace sampler and GCMS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	External device
Injection Location	Front
Mass Spectrometer	Enabled

6890 GC METHOD

OVEN

Initial temp	38 °C (On)	Maximum temp	240 °C
Initial time	5.00 min	Equilibration time	2.5 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	200	5.00
2	0.0(Off)		
Post temp	200 °C		
Post time	0.00 min		
Run time	20.8 min		

		<i>Front Inlet</i>
Mode		Split
Initial temp		175 °C (On)
Pressure		1.23 psi (On)
Split ratio		8:1
Split flow		11.9 mL/min
Total flow		16.1 mL/min
Gas saver		off
Saver flow		
Saver time		
Gas type		Helium

	<i>Column 1</i>	<i>Column 2</i>
Capillary Column		(not installed)
Model number	Restek RTX-BAC1	
Max Temperature	240 °C	
Nominal length	30.0 m	
Nominal diameter	320.00 µm	
Nominal film thickness	1.80 µm	
Mode	constant flow	
Initial flow	1.4 mL/min	
Nominal init pressure	1.24 psi	
Average velocity	43 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

<i>FRONT DETECTOR (NO DET)</i>	<i>BACK DETECTOR (not used)</i>
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

<i>Post Run</i>	
Post Time	000 min

Time	Specifier	Parameter & Setpoint
7673 Injector	Not used	
<i>Front Injector:</i>		

Sample Washes	0	
Sample Pumps	0	
Injection Volume	3.0 microliters	
Syringe Size	10.0 microliters	
PostInj Solvent A Washes	0	
PostInj Solvent B Washes	0	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
PreInjection Dwell	0.00 minutes	
PostInjection Dwell	0.00 minutes	
<i>Back Injector:</i>		
No parameters specified		

MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	lomass.u
Acquisition Mode	Scan
<i>MS Information</i>	
Solvent Delay	1.55 min
EM Absolute	relative
EM Offset	200
Resulting EM Voltage	(varies)

[Scan Parameters]

Low Mass	29		
High Mass	175.0		
Threshold	150		
Sample #	2	A.D Samples	4

[MSZones]

MS Quad: 150 °C maximum 200 °C
MS Source: 230 °C maximum 250 °C

END OF MS ACQUISITION PARAMETERS

The Agilent Headspace Sampler Parameters are as follows:

Matrix	
Matrix	Blood
Matrix Boiling Point	100.0 °C
	Temperature (°C)

Sample Oven	65.0
Sample Valve	100.0
Transfer Line	100.0
	Time (minutes)
GC Cycle	45.0
Sample Equilibration	5.0
Vial Pressurization	0.20
Loop Fill	0.20
Loop Equilibration	0.10
Sample Inject	1.0
Oven Stabilization	0.5
	Shaking
Agitation	Low
	Parameter
Oven Step	No
Equilibration Time	No
	Mode
Extractions	1
Puncture Mode	Single

INSTRUMENT SETUP

Perform a Mass Spectrometric (MS) Tune to check if the instrument is operable, using the low mass autotune procedure. See the "Mass Spectrometric Tuning (Autotune)" SOP for specifications.

An acceptable autotune must be obtained prior to batch analysis.

Prepare a sequence using the following steps.

When Chemstation is opened, the **Openlab ECM Login** screen appears, Enter the instrument name (ms3, ms4, etc.) as appropriate for username and the current password. Verify that Account field says production and Domain field says Built-In. if Chemstation is already running, it may be necessary to log out and relog in. Using the Chemstation software, at the top Method and Run toolbar under **ECM**, select **Logon to ECM**. Follow the instructions above to log on.

1. On the Method and Run toolbar, under Sequence, select Load Sequence. Select default.s. Click on Select.
1. In the Method and Run toolbar, under Sequence, select Edit Sequence. At the top of the screen under Data Path, click on Browse. Under Select Data Path, click on the msdchem folder. Then click 1 and then click on the Data folder to highlight it. In lower left of dialog box, select Make New Folder. A folder with the name New Folder is created under DATA. Right click on New Folder and Rename or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS3041111a. Click OK which will take you back to the sample log table.
2. In the Sample column verify the correctness of pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-11-2432 fem). If the sample has been diluted, enter the appropriate dilution factor in the Multiplier column. For QC samples use an appropriate designator. Enter the next sample in the batch in the next open field down the column.
3. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration or QC.
4. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct.
5. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
6. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS20411a001. Click on this cell, hold down the left mouse and drag to the last vial number of the sequence (the cells will be highlighted). Right click and select Fill Column and Increment. Verify that the data file information for each vial is correct.
7. In the Comment column, enter any additional information for the vials.
8. In the Multiplier column, enter any sample dilutions.
9. In the Level column, verify that the correct level numbers are entered for calibrators in this batch.
10. Verify No Update is selected for all vials under Update Rf and Update Rt.
11. Review the information typed for the sequence. Correct any information as needed. Verify that the Data Path is C:MSDCHEM\1\Data\current sequence name. Verify that the Method Path is C:MSDCHEM\1\METHODS. Then Click ok.

Note: Occasionally, it is desirable to run several subsequences in one batch. Use the instructions below to accomplish this.

Setting-Up a Subsequence

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence i.e.: **C:\MSDCHEM1\DATA\MSNMMDDYYx** where "N" is the instrument's number and "x" is the letter designated to the subsequence (**it must be different than that of the original sequence**).
4. The suffix of the data files must be different from that of the original; i.e.: MSNMMDDy001; **the subsequence data files must start with 1 again**.
5. After typing in the entire sequence, save sequence accordingly.
6. Go to Sequence → Simulate Sequence → Run Sequence.
7. A dialog box will pop-up: DataPath **C:\MSDCHEM1\DATA\MSNMMDDYYy** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
8. A 2nd dialog box will pop-up: Create **C:\MSDCHEM1\DATA\MSNMMDDYYy**? → Click **Yes**.
9. A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.

Setting-Up a Subsequence with a Different Method

On the Sample Log Table:

1. After inserting the DataPath keywords and Commands, Insert a Row.
2. Select "**Keyword**" for "Type"
3. Select "**MethodPath**" for "Method/Keyword"
4. Check that the method for each sample is changed to the new method.
5. Follow Steps 5-9 in the "**Setting-Up a Subsequence**" Section (see previous section).

12. On the Method and Run toolbar, under Sequence, select Run Sequence. In the dialog box under Sequence Comment enter the initials of the individual who has entered the sequence in the Operator Field (e.g., MPM). Under Data File Directory verify that the data file path is C:MSDCHEM\1\DATA\batch name.
13. Under Sequence, select Save Sequence As. Under File Name, type in the name of the folder that the batch will be saved to under DATA, MSNMMDDYYx (e.g. MS2041211a) Select Save. The extension “.s” will automatically be added.
14. Under Sequence select Print Sequence. Verify that Brief Format is selected and click on OK. The sequence will be printed. Apply the preprinted labels for documenting verification of process steps to the printed sequence list.
15. Use the printed sequence list to load vials into the appropriate autosampler positions as indicated by the order on the printed sequence list. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler as indicated on the sequence list. Document correct positioning by dating and initialing the appropriate line on the sequence list.
16. Under Sequence select Run Sequence. Verify that the Sequence comments and Data Field information are correct (i.e. verify that the proper sequence is loaded. If not, load the proper sequence). Click on Run Sequence.
17. After the batch is finished, unload the vials. Compare the vial information to the sequence list as they are removed, to verify that the correct vial was in the correct position. Date and initial the sequence list when this is completed. Annotate discrepancies if necessary

DATA TRANSFER AND PROCESSING

All processing and review are performed on a processing computer.

SAVE METHOD TO ECM

1. After the run finishes, the data files will be in the data subdirectory on the local chemstation and also automatically transferred to ECM. From the acquiring computer, make sure the proper method, the one used to acquire the data, is loaded. On the top toolbar under ECM click on Save Method to ECM.
2. Click on the GCMS, the correct instrument name folder, the appropriate month and batch to which the method will be saved.

RETRIEVE BATCH FROM ECM

1. At the processing computer, click on Processing Data Analysis. Log on using your OCME network username and password.

2. On Enhanced Data Analysis screen, click on ECM at the top toolbar and select Retrieve entire sequence from ECM.
3. This will open up Openlab ECM screen. Select GCMS, then the appropriate instrument, the month, and, finally the batch to be retrieved. On the status line at the bottom of the screen that the batch is being retrieved. The batch will be downloaded to the following location: C:\msdchem\1\ECM\Retrieve\”batch name”

PROCESSING USING ENHANCED DATA ANALYSIS

On any processing station:

1. Click on the Processing Data Analysis icon on the desktop and Log-in by using: analyst's ocme login for the username and their password.
2. From the tool bar menu select **ECM → Retrieve Entire Batch From ECM**. Browse to locate and select the batch to be processed. When the proper batch is highlighted, click OK to start the retrieve. Each datafile will be retrieved along with the method to be used for processing. The progress of the retrieve will show on the bottom line of the screen. Verify that the sequence and method are the correct files that will need to be processed.
3. Once the retrieval is complete the batch folder is located in the C:\msdchem\1\DATA\ECM\retrieve folder.
4. Select the method under the batch by right clicking on it and **Load** the method. Select the Calibrator 1 data file in the batch (usually the first one), right click, and select **Load** to load the data into memory.
5. In the white area just below the bottom icon bar, type QT 1, then click the execute button to the left of the white area. This will quantitate the data using the loaded method, but will not produce a report. Next click on the QEDIT icon (or under view in the menu bar, select QEDIT Quant Result). Four windows will be presented. On the far left, a list of all compounds in the calibration table starting with the compounds, center and right on top, the extracted ions of the compound selected, center bottom, the spectra of the selected peak, and bottom right, information about the compound selected from the list to the left.
6. Double click on the internal standard, the first compound in the list. Review the integrated baseline to confirm that the correct peak for the compound has been selected and the extracted ion chromatographs were properly integrated. Redraw baselines if necessary. If the wrong peak was selected, draw baselines under the correct peak in the ion chromatographs. Double right clicking in the mass spectrum window will perform a library search of the spectrum displayed. Use as necessary to check peak identity. Double clicking on the compound names marked with a star will load that compound for review
7. Repeat step 6 for each compound in the calibrator, following the same criteria as listed in step 6. When finished, click exit. A window will appear. Select yes to “save changes to quantitative results?” if no changes to the compounds were made, it may be appropriate to select no. Think about what you want to do, and answer appropriately.
8. When all compounds in the calibrator have been reviewed, click on the Update Calibration icon (graph with yellow up arrow, in the middle right of the second row of

icons) to update the single level of calibration. A window appears. Make sure "Update one level" is selected, and click OK. A window appears, saying "file has previously been quantitated. Requantitate now?". Select no. Then the Update Calibration window appears. Click on "Update Level (select existing Calibration Level ID)", so that a black dot appears in the white circle. Then select Responses, Replace, Retention Times, Replace and Replace Qualifier Relative Responses. Highlight 1 in the Existing Level ID box. Click on Do Update button. This will update the calibration for level 1 based on the calibrator just reviewed.

9. The Edit Compounds screen appears. Click on OK. A window appears asking "current changes will be saved. Proceed?". Click OK. This produces a calibrated method in memory
10. In the menu bar select method. Select save method. A box appears, showing the path to the method. Check to make sure the method path is correct, leading to the method under the current batch that is being processed. Another box appears, with a browser in ECM. Click cancel, as the method will be saved to ECM later, after printing is completed, and after all changes are made.
11. Now all the files in the batch being processed must be quantitated against the calibrated method. On the menu toolbar, click on Tools and then click on Dolist. The Qlist Configuration window will appear. Under the Qlist Configuration window. In the left hand window are various options. In the Selected Options window on the right, the only program listed should be Quant No Report. If any other option(s) is present, click to highlight it and select remove. If Quant No Report is not present in the Selected Options box, choose Quant No Report from the left side by clicking on it and then select Add. Verify that when Quant No Report is highlighted in the left side box, the command string line at the bottom of the window shows qt 1. Finish by selecting OK.
12. Under the Multiple File screen, verify that the correct batch is loaded. If the correct batch is not loaded, load the correct batch by selecting Change Path, then selecting the batch to be loaded. Select OK. Verify that the path for the data files is correct.

Note: Correct path is C:\msdchem\ecm\retrieve\current batch name

13. In the left "available data file" screen, highlight all files and select right arrow to load them into the Files Selected for Processing window. Click Process. The entire batch will be quantitated.
14. To review the processed data files on the "Data Analysis" screen, select the Calibrator 1 data file in the batch (usually the first one), right click, and select Load to load the data into memory. Visually examine the chromatographs for significant peaks, especially peaks that may not be compounds in calibrator 1. By right double clicking on the apex of a peak, the mass spectrum of that point will be shown in a window. Right double clicking on a point at the leading edge of the peak will load that spectrum. By clicking on the subtract icon (11th from the left in the second row of icons), a background-subtracted mass spectrum is displayed. This spectrum may be library searched by right double clicking in the spectrum window. The libraries searched and other criteria can be adjusted by clicking on the icon immediately to the right of the subtract icon.

15. Click on the QEDIT icon (or under view in the menu bar, select QEDIT Quant Result). Four windows will be presented. On the far left, a list of all compounds in the calibration table starting with the mass spec compounds, then the NPD compounds. Center and right on top, the extracted ions of the compound selected. Center bottom, the spectra of the selected peak, and bottom right, information about the compound selected from the list to the left.
16. Repeat steps 6 and 7 above for each compound marked with a star, and for any compounds identified in the chromatogram review step, until all compounds present have been identified (marked with a star in the Qedit list). When finished, click exit. A window will appear. Select yes to "save changes to quantitative results?".
17. On the menu bar, click Tentatively ID, then LSC Summary to Screen. This will integrate the TIC, subtract any peaks marked as identified in Qedit, select the 15 largest peaks remaining, perform a background subtract, and then perform a library search on each peak. When this is done, click Tentatively ID, then Edit LSC results. a window will appear, showing the mass spectrum of the first peak, and the library spectrum of the best hit. Compare the two spectra. If there is a match, click next, and the next spectrum will be displayed. If there is no match, click delete, then next.
18. To print reports, select Tools from the toolbar, DoLIST, full quant w/o calculations (QT 0,1,`F'), and detailed LSC w/o calculations (Report_TIC 0,1,`F'), Add, and OK. Select files to print, click the → Arrow and Process.
19. Save files to ECM. Select ECM from the toolbar, select "Save multiple data files to ECM". Select all files.
20. Save method to ECM. Select ECM from the toolbar, Save Method to ECM. Make sure data path is correct.

BATCH CLEAN UP

1. Select my computer. Find the batch on the C drive at C:\msdchem\1\ecm\retrieve\batch. Right click on the batch to be deleted and select delete. Do not delete a batch that has not been successfully uploaded to ECM.

ACCEPTANCE CRITERIA

1. Peaks must be symmetrical.
4. The negative control (blank) must have no interfering peaks in the target areas of interest.
5. Satisfactory baseline resolution of all peaks of interest is required.

6. The “pattern” of the scan of the peak in question must match the “pattern” of the reference or library scan. It is difficult to give objective criteria for a “pattern” match. Most of the significant ions in the reference scan should be present in the sample scan. Any doubts regarding the correct identification, due to the complex nature of mass spectra, should be discussed with a supervisor.

REPORTING

1. Chromatograms, along with a copy of the sequence worksheet and all controls, are entered in each appropriate case file. The sequence worksheet will indicate the analyst who loaded/ removed the samples from the headspace and whether the batch has met the minimum acceptable criteria of the laboratory. All control results must be entered in the QC/QA database, if applicable. In the event a batch has not met the minimum acceptance criteria, an explanation must accompany the failed batch and the chromatogram(s) must be filed with the relevant cases. The case must be rescheduled, and the appropriate aliquot number cited.
2. All negative cases are reported as “not detected” on the toxicology report sheet.
3. Compounds positively identified are reported as detected. Compounds identified only by library match are reported “(library match only)”.

REFERENCES

The Merck Index. An Encyclopedia of chemicals and drugs. Ninth Edition. Published by Merck and company, U.S.A.

James C. Garriott, ed., *Medicolegal Aspects of Alcohol Determination in Biological Specimens*, Lawers & Judges Publishing Company, Tucson, AR, 1993.

Feiser and Feiser. *Organic Chemistry*. D.C. Heath and Company Boston. Second Edition

Agilent Headspace Sampler Manuals.

Agilent 6890 Series II Gas Chromatograph Operating Manual and Reference Manual

Agilent 5973 & 5973 Network Mass Selective Detector Installation Guide.

Agilent 5973 & 5973 Network Mass Selective Detector Users Guide.

Agilent 5973 & 5973 Network Mass Selective Detector Standard Operating Procedures.

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