

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK**

**GC/MASS SPECTROMETRIC TUNING
(AUTOTUNE)**

PRINCIPLE

All quadrupole mass spectrometers are tuned with a compound of known mass spectrum (PFTBA - perfluorotributyl-amine for our instruments) to insure that the instrument is operational and has a similar response to other instruments. All instruments are tuned prior to use each day batches are initiated. The autotune program adjusts the many parameters of the source, mass filter, and detector to give a consistent response to the tuning compound over the entire mass range, with excellent sensitivity and accurate mass assignment. Thus, the resulting spectra are consistent from instrument to instrument and from day to day. This also allows mass spectral libraries to be used for searching unknown peaks and confirming known peaks. The autotune program uses three ions from the PFTBA spectrum for its tuning: m/z 69, 219 and 502. The program also uses the abundance of the naturally-occurring C¹³ isotopes at 70, 220, and 503 as a check of tuning.

AGILENT 5973 GC/MS

1. Double click MSTOP icon in windows. This will load the instrument panel.
2. On upper left pull-down menu, click on "Instrument".
3. Move down menu and highlight "Perform MS autotune".
4. In Select Tune Type pop-up box select "Autotune".

Note: *Autotune is selected from the three options (Autotune, Standard, Quicktune) because it is the most comprehensive tune. Standard tune sets standard response values over the mass range. Quicktune only adjusts EM voltage and peak width; there are no lens corrections made, which is not sufficient for our purposes. Autotune maximizes instrument sensitivity over the entire mass range and makes corrections as needed. This is most suited for our purposes.*

5. Click on OK. Autotune will proceed until completion and print out an autotune report.

AUTOTUNE ACCEPTANCE CRITERIA

1. The m/z 69 is usually the base peak (100%). After a new or freshly cleaned source is installed, the m/z 219 peak may become the base peak for autotune. This is acceptable. If the m/z 219 peak becomes the base peak, the m/z 69 peak must be greater than 70% of the m/z 219 peak.
2. The m/z 219 must be greater than 40% of the m/z 69 peak (except when 219 is the base peak (see # 1 acceptance criterion).
3. The m/z 502 must be greater than 2.5% of the m/z 69 peak

Note: Any values greater than the target values of 100%, 40%, 2.5% constitute acceptable ratios.

4. Isotope ratios should be $\pm 20\%$ of target values (1.08 ± 0.216 for m/z 70, 4.32 ± 0.864 for m/z 220 and 10.09 ± 2.01 for m/z 503).
5. The m/z 28 (nitrogen, "air") and m/z 18 (H₂O, "water") must be less than 10% of the base peak.

Note: If the m/z 28 is greater than 10% the system is "leaking", i.e., air is getting into the system. This must be corrected prior to injecting samples.

Additional considerations for a proper tune are:

- a. EM volts should vary no more than ± 150 volts of the previous tune. A larger variation than this indicates that the source may need maintenance.
- b. Filament emission should be set at default of 34.6 μ A.
- c. Electron energy should be set at default of 70 EV.
- d. DC polarity should be as the factory recommends, and Ion Polarity should be positive.
- e. Foreline pressure should be around 50-60 mTorr for diffusion pump instruments. For turbo pump instruments, turbospeed should be 100%.
- f. Ion Focus volts should vary around 80 (60-100). A larger voltage than this could indicate that the source needs maintenance.

Gain, offset, entrance lens, x-ray, mass gain, and mass offset values should vary by no more than 20%. Any variance greater than this could indicate that the source needs maintenance. Criteria 1 through 5 **must** be met for the tune to be accepted and specimens run. If any of these criteria are not met, the autotune must be repeated. Occasionally, the subsequent tune will be better or the tune will return to previous values. The system is ready to run when the tune is acceptable. Two consecutive autotune fails require system maintenance before any specimens are run. Criteria *a* through *g* are transient values. Changes in these variables greater than those stated above do not necessarily constitute a failed tune. These parameters must be viewed overall and, in the case of extreme changes, a decision on tune acceptability is made. In addition, after the profile scan is run, evaluate the symmetry of the peaks used for the autotune. Any peak splitting could be a sign that the source is in need of cleaning. If peak splitting is more than 10%, the system should not be used until source maintenance and/or cleaning is completed. Consult with the supervisor or a manager if in doubt.

LOWMASS AUTOTUNE

Some procedures benefit from a reduced linear range in mass acquisition. For these methods, use a low mass autotune. This is an autotune using m/z 69, 131, and 219, rather than m/z 69, 219, and 502. The shorter linear range offers an increase in sensitivity for low molecular weight compounds.

1. From the top menu bar of the Instrument Control screen, click VIEW, then TUNE AND VACUUM CONTROL..., the screen changes to TUNE.
2. Click FILE in the menu bar, then LOAD TUNE PARAMETERS. Select ATUNE.U, then click OK. The previous autotune parameters are loaded into memory. On the menu bar, click TUNE, then click AUTOTUNE (ATUNE.U). A normal autotune is performed, and a report printed. Review as in Autotune acceptance criteria above. Click FILE in the menu bar, then SAVE TUNE PARAMETERS. Select ATUNE.U, then click SELECT. This saves the parameters to ATUNE.U. If autotune fails, proceed as above.
3. On the menu bar, click TUNE, then click LOW MASS AUTOTUNE (LOMASS.U). The low mass autotune is performed, and a report generated. Use the same criteria as above, except that the 502 criteria do not apply, and the 131 m/z should be greater than 30 %, with the 132 isotopic ratio $3.6 \pm 20 \% (\pm 0.72)$
4. Click FILE in the menu bar, then SAVE TUNE PARAMETERS. Select LOMASS.U, then click SELECT. This saves the parameters to LOMASS.U. If low mass autotune fails, proceed as above.
5. Click VIEW, and select INSTRUMENT CONTROL. A warning appears asking if the tune file has been saved. If you did not save the tunes into the proper files, click NO, otherwise, click YES.

REFERENCE

Agilent 5973 MSD Reference Collection CD and manuals.

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