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Optimizing Conditions for Volatiles Analysis

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OPTIMIZING CONDITIONS FOR VOLATILES ANALYSIS

Over the years, many configurations for Volatile systems have come and gone. Now virtually all labs run a narrow bore column and split/splitless injection port. Thus, we will address only this configuration.

The most common narrow bore columns are 30-60 meters long, 0.18 mm or 0.25 mm ID. These are the considerations with this configuration.

- The connection from the Purge and Trap to the split/splitless injector is CRITICAL. Over the years, we have come to the following conclusion:
  - For 5890 GCs (regardless of the MSD involved), you are better off using the "direct insertion technique" whereby the transfer line of the purge and trap is butt-connected to a piece of 0.32 mm ID fused silica which is then inserted directly into the injector, thereby leaving the split/splitless pneumatics intact. This configuration not only leads to better chromatography but reduces exposure of the analytes to the stainless steel in the injector.
  - For 6890 GCs (regardless of the MSD involved), you are better off using the "slice and dice technique" whereby the transfer line from the purge and trap concentrator is teed into the stainless steel lines of the split/splitless injector.
- Also, be sure to use a gold inlet seal and a 1 or 2 mm injection port liner for 5890s and either a 2 or 4 mm liner for 6890s.
- For 5890s - desorb flows between 25-30 mL/minute are ideal whereas with the 6890 performance is usually better at 30-35 ml/minute.
- Pressure pulsing usually provides good results, with initial head pressures of 25-30 psi for 1 minute and then a constant flow of 0.4-1.2 mL/minute depending on your column and MSD. For 0.18 mm ID columns a flow of 0.4 mL/minute is a good starting point while for 0.25 mm ID columns a flow of 1.0 mL/minute is a good starting point.
- Monitor Source pressure. The pressure should always be in the low 10^-5 to high 10^-6 range with this configuration. Monitor and record the source pressure with the column flow set to what it typically is in your analytical run and keep a record of it. If problems occur, note the changes in source pressure.
- Desorb with shorter desorb times. I recommend a 2 minute desorb time for virtually all VOA applications.

Optimizing Purge and Trap Conditions

Regardless of how your GC/MS is configured, you need to optimize purge and trap conditions. At this point in time, virtually all discussions about Volatiles analyses relate to purge and trap concentrators using the VOCARB 3000 trap.

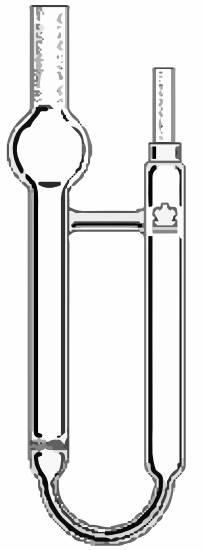
"Standard VOCARB 3000 recommended conditions" for most 500, 600 and 8000 series Volatile GC/MS methods are as follows:

Sample temperature:	ambient (aqueous) or heated (non-aqueous)
Purge:	11-15 minutes at 40-45 mL/minute
Dry purge:	2-4 minutes
Desorb preheat:	220° C
Desorb:	2 minutes at 230° C
Bake:	7 minutes at 260° C

To optimize the system, the analyst can vary any of the above parameters based on the types of analytes in the method and the configuration employed. The following illustrates important considerations:

Purge Conditions

You must consider both flow rate and time.



Both parameters will have DRAMATIC effects on recoveries of certain analytes. Generally 11-15 minutes at 42-45 mL/minute often works best if the method requires monitoring of Volatile gases. Longer times and higher flows help the more water soluble analytes, and shorter times and lower flows help the more Volatile analytes. You will usually obtain better results by purging at a lower flow rate for a longer time rather than cranking the purge flow really high and reducing the purge time.

The use of Dry Purge (generally 2-4 minutes is sufficient) is critical to controlling water vapor buildup on the trap.

Desorb Conditions

Key points:

1. Desorption efficiency ranks at the top of the list as to what affects chromatography so pay close attention to desorb flow, desorb time and desorb temperature!

   Desorption efficiency is the term that expresses how well the analytes are transferred from the trap to the GC inlet. The goal is to focus the analytes in a tight band on the trap and then transfer them in that same tight band to the GC inlet. A tight band of analytes into the inlet leads to the best possible separation and peak shape.

2. For Tekmar systems, it's usually best to desorb Preheat at 5° C below the desorb temperature so that the trap will be fully heated before cycling the 6 port valve.
3. Desorption efficiency is highly dependent on the desorb flow rate. Low desorb flows of 10-25 mL/minute are usually not as efficient as high flows of 25-40 mL/minute but you should try various desorb flows to see what works best.
4. Generally, the higher the desorb temperature, the sharper the peaks will be. However, thermal degradation of certain species can occur (a good starting point is 230° C).

Bake conditions

Key point:

Bake does not affect chromatography except to minimize cross-contamination and carryover. My recommendation is to bake for 7 minutes. Use Bake Gas Bypass with a 60 second delay if your system is so equipped (unless you have a single-spot Purge and Trap with the Auto-Drain feature in which case you do not use Bake Gas Bypass). This way, the bake flushes pure helium through the trap (heated to its maximum temperature) and reduces carryover. Longer bake times will reduce trap lifetime, but whether the trap lasts 2 months or 3 is not nearly important as keeping the system as clean as possible.

General Guidelines for Volatiles

A "good" ICC is one that meets the following criteria:

1. LINEARITY: Has low RSD's (relative standard deviations) for most or all compounds. This means the system is linear throughout your working calibration range which translates directly into accuracy of data.
2. SENSITIVITY: Has adequate response at the *low* standard to meet the stated detection limit. The push with all state and federal methodologies (especially 500 series drinking water and most 600 series wastewater) is for lower MDL's, so being able to detect low levels is critical.
3. REPRODUCIBILITY: In addition to being linear and sensitive, the system must be reproducible. After you run your multi-level curve, your daily (or 12 hour) check standard should also yield low RSD's-indicating *minimal* drift from your ICC. A properly optimized system should stay linear for at least two weeks. If you need to recalibrate more frequently than once every week, your system is not optimized.

The following guidelines will help you keep your system linear and sensitive:

- Make sure the system is tuned reproducibly each day. Keep your relative ratios and peak widths of ions 69, 131 and 219 in your tune file constant from the time you set up your initial calibration curve to your day to day continuing calibration check. I recommend making a hard copy of a Spectrum Scan and Profile Scan when running your initial curve. Each day, try to attain the same ratios, peak widths and sensitivity. Keeping your system tuned each day will go a long way in keeping your system linear.
- Make sure the Purge & Trap is cleaned thoroughly. The type of system you have will determine how you clean it, but in any case, you should flush the system out with clean helium for 2 hours each 24 shift prior to running anything.
- Monitor spot-to-spot variations in flow rates of your autosampler. If you have an automatic sampler that has different ports for each sample (i.e. ALS-2016), you should measure the flow rate from each spot, because the valve in these systems sometimes gets out of alignment from use, and can vary the spot-to-spot purge flow rate, which would cause reduced linearity and reproducibility. If the flow varies by more than 4 mL/minute, the valve should be realigned or replaced.
- Monitor the areas of the internal standards and adjust the electron multiplier as necessary. Record the internal standard areas you obtain when you run your initial curve, and increase or decrease the multiplier during the subsequent weeks in order to keep those internal standard areas as close as possible to what they were when you ran the initial curve. In other words, keep the ISTD areas from drifting. Adjust the EM setting daily to make sure they stay constant. Daily monitoring and adjustment of electron multiplier voltage is frequently necessary and essential to staying linear for extended periods of time.
- Beware of active sites. Unless you are running drinking water samples, it's inevitable that sample contamination will create active sites to some extent. Periodic methanol flushing lines and valves is helpful, but replacement of the 6 port valve and sample lines on the Purge and Trap on an annual basis is a good idea.
- Beware of excess acidity which can cause 2-Chloroethyl-vinyl ether to break down.
- Beware of excessively high desorb temperatures which can cause certain analytes to break down on the trap during desorb.
- Be sure you are using the correct EM voltage. Sometimes, you need to increase it above what you used in manual tune, depending on your configuration. Use the following table as a starting point (Note: these are just guidelines, the overall quality of your curve determines the proper EM setting). Most laboratories aim for about 2 million counts of ion 69 in manual tune (assuming Enviroquant Version G1701BA or equivalent)

Configuration	Usual EM Voltage Guide
0.25 mm ID capillary column w/split i.p.:	Manual Tune +200 volts
0.18mm ID capillary column w/split i.p.:	Manual Tune +300 volts

Once you get a good ICC, record the area of the first internal standard and shoot for the EM voltage that gives you that same area and you should be able to reproduce your results!

- Be sure you are using the correct A/D sampling rate. This stands for Analog to Digital setting and is a measure of how many scans the system will average before storing a single spectrum point. When you view a TIC (Total Ion Chromatogram), you are actually seeing a big "connect the dots" picture. Each point on the TIC is one spectrum, and each spectrum is actually the average of several scans. The number of scans in a TIC is a function of 3 things:
  1. The scan range being used (typically 35-300 amu).
  2. The model of MSD. The newer 5973 and 5975 models are faster than the 5971 and 5972's which in turn scan faster than the 5970 MSD.
  3. The A/D setting. A/D is expressed as an exponent of 2; i.e. 2^1=2; 2^2=4, etc. The larger the resulting A/D the more scans get averaged before a point is stored and thus you collect less data points. Use the sampling rate that gives you 10-20 scans across the peak. If are you getting less than 10 scans, that means you need to lower the A/D to increase the scans. Conversely, if you are getting more than 20 scans across the peak you need to raise the A/D to decrease the number of scans.
- Be sure you are using the correct threshold. This is the "all-or-nothing" level. Abundance counts of ions below this level register as 0; abundance counts of ions above it get stored with their corresponding abundance in that scan. You should set it low enough to see all minor ions at your detection level.

  Here's how to set the Threshold: you need to do a test run in which you set the Threshold to 0 in your method. Run a low level standard (let's say 2.0 ppb for 8260 or 0.5 ppb for 524). You will get a chromatogram with a noisy baseline (this is expected). Look on your Quant report and determine the 10 poorest responding compounds. Then do a spectrum scan at the apex of each of those 10 analytes whose secondary or tertiary ions are at low percentages (i.e. <20% of the base peak). Obtain a tabulation of the abundances. Find the smallest result.

  Set your threshold to 1/2 the abundance of the smallest qualifier ion in one of your 10 smallest peaks in your low level standard. This is a good setting which enables you to see all minor ions and eliminates as much background noise simultaneously.

- Consider using pressure pulsing followed by constant flow. Pressure pulsing is where the head pressure is raised at the time of desorb then lowered to facilitate constant flow throughout the balance of the run. Example using a 0.18 mm ID column with a 2 minutes desorb:
  - You set the head pressure to 30 psi for 2 minutes to match the desorb time.
  - After 2 minutes you set the program for constant flow thereafter.

Benefits of this setup

1. The high head pressure during desorb helps focus the water/methanol/analyte slug coming from the Purge and Trap during desorb into a tight band entering the column. That is a natural follow up to optimizing desorb conditions to which I referred earlier.
2. The constant flow for the balance of the run directly translates to constant ion source pressure in the MSD. Constant ion source pressure is extremely helpful in maintaining pump integrity and keeping the GC/MS stable over long periods of time.

Keys to Optimizing the Quantitation Routine

For you DOS users with Enviroquant here are my tips on optimizing the compound list:

1. Retention time window: I recommend 1 minute. That is ample time for the integrator to properly integrate.
2. Integration parameters: Use 5000 area counts for most compounds. Start threshold = 0.200; stop threshold 0.000. Data point sampling = 1. Smoothing box checked. Detection filtering = 5 points. Don't forget to set your MDL cutoff in "Edit Quant Report Options."
3. Curve fit: Average of Response factors.
4. Subtraction method: Use no abundance subtraction. I've found that the other methods are needed only if poor chromatography exists.
5. Identify by: Use Combination Q value and retention time. This means that if there are multiple hits in the RT window, the system will choose the correct one based on both Q value (i.e. how close the ion ratios are in the peak as compared to the ID file) *and* retention time. This is the best way.
6. Relative response: 50% Relative (NOT absolute). This may seem wide (and indeed it is) but I can always delete false positives. The last thing we want to have happen is a false negative. It is better to widen the window and delete a mishit, than to have too narrow a window and miss a compound that is present!

Key points to maintaining an effective Compound List:

- ALWAYS have at least two ions for each compound. Once you set up a good compound list, DON'T start cutting out ions if the system fails to locate the compound. Find out why and correct. In other words, don't butcher your compound list; instead constantly fine-tune and update it as your chromatographic and spectral changes warrant.
- Update RT's and Q values daily. You can do this automatically in "Update Levels". Also update reference spectra daily.
- Use as many qualifier ions as possible. If the compound produces 4 qualifier ions that have >20% response, then enter them all. Use ions throughout the entire mass range rather than using several ions with similar masses.
- Modify the global parameters listed above on a *compound by compound* basis for the most effective quantitation. The compound list is the heart of accurate quantitation and qualification for target analysis; do your best to keep it optimized each day! Create a customized integration parameter file for any compound which cannot be accurately integrated by the default parameters.

Questions or comments on this or any issue of OPTIMIZE may be emailed to the author, Mark Ferry, at MFerry@SPEX.com.

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