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Optimizing Conditions for Semivolatiles Analysis

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OPTIMIZING CONDITIONS FOR SEMIVOLATILES ANALYSIS

Basically, the configuration to run Semivolatiles for EPA Methods 625/8270/525 is relatively simple: we employ the split/splitless injector, and a narrow bore capillary directly interfaced into the Mass Spectrometer.

Below are the key parameters and a discussion about each:

Column Selection:

The stationary phase for the fused silica capillary column used for these methods is a 5%-diphenyl-95% dimethyl polysiloxane (e.g. DB-5; Rtx-5; SPB-5, etc.). I have also used SGE's SolGel 1 column which is 100% dimethyl polysiloxane and have obtained similar results with both columns.

Generally, the column is 30 meters long. Also, although 0.32 mm ID can be used on H-P/Agilent benchtop mass specs, our many years of experience have led us to conclude that the 0.25 mm ID column should be used most of the time. The flow with the 0.32 column is dangerously high and although it can be made to work, the potential risk to Turbopump and Oil Diffusion pumps systems is too great to justify its usage. In addition, the higher head pressures associated with 0.25 mm ID columns give us more flexibility in pressure pulsing.

Thus, the phase, ID and length are pretty much predetermined. However, the analyst has a choice regarding the F.T. (film thickness). Choice of this parameter is based on 3 main criteria:

1. Calibration range used
2. Sample capacity needed vs. run time desired
3. Nature of analytes and method being employed.

There are basically three film thicknesses from which to choose: 0.25 µm, 0.50 µm and 1.0 µm. As the film thickness increases, the following occur (and vice versa):

1. Run times increase
2. Sample capacity increases (i.e. overload occurs less often)
3. The potential for column bleed increases
4. Peaks become broader
5. The column lasts longer

Thinner films produce sharp peaks and short run times with the cleanest baseline. Thicker films are better suited for "loaded" samples (i.e. those with high amounts of non-target analytes, such as hydrocarbons). For most profit-minded commercial labs, use of 1 µm film is not recommended unless EPC is present, otherwise the run times are prohibitively long. It's really a toss-up whether to use a 0.5 µ FT column or a 0.25 µ FT column.

Column Installation

After you choose your column, you then need to install it into the GC/MS. Be sure to follow these guidelines when installing a new column:

1. Install the column into the injection port and let helium flow through it at ambient temperature for 30 minutes. I recommend a 5 mm column insertion distance above the top of the ferrule into the injector.
2. With the column out of the detector (which should be capped off), slowly ramp the column to its operational maximum temperature and hold it there for about one hour.
3. Cool the oven and install into the detector. For the 5970, 5971 and 5972 MSDs, install the end of the column into the MSD until it butts against the analyzer, then pull back about a half inch. You may have been told to pull it back only 2 mm to minimize dead volume, but the capillary direct interface is under high vacuum, so I like to leave a little more distance (i.e. half an inch) to minimize the chances of contact between the column and analyzer parts which would short out the system.

   For the 5973 MSD, the column needs to be installed such that it lines up exactly even with the end of the transfer line. When you slide open the analyzer door, you slide the analytical column until you see it protrude from the transfer line, then back it off until it is flush with the transfer line. If the column extends more than a few mm beyond the transfer line, it can short out the source.
4. Pump down the Mass Spec and condition the column as usual.

Heated Zones

There are only two heated zones to set: the Injection Port and mass spec interface. Use the Agilent recommended settings for the mass spec interface leaving the Injection Port to be user-determined. I recommend 250°C for the Injection Port and be sure the insulator is present, otherwise response for the high molecular weight analytes will be poor. Some labs like to run the injector hotter (around 280°C) but breakdown of the aniline compounds can occur so I strongly recommend 250°C with the insulator.

Pressures and Flows

There are two flows (Total Flow and Septum Purge Flow) and one pressure (Column Head Pressure) which you need to set. I'll give you my recommendations for systems with and without EPC.

Total Flow: 43 mL/min. This is the total flow into the injection port which splits into 3 directions: a small amount, generally 1.0 mL/min, and based on the head pressure and size of the column enters the column and serves as the carrier flow; another small amount, generally 2 mL/min hovers, under the septum and out the Septum Vent; the majority of the flow, about 40 mL/min, sweeps through the injection port and out the total flow vent.

Septum Flow: 2mL/min. This is not critical. For systems with EPC, it is pre-set for you. It's designed to rid the system of any septum bleed.

Head Pressure: (see chart below)

Column Size	hp (psi)	Resulting Flow (approx)
0.25 mm ID (without EPC)	12	35 cm/sec *average* which equals about 1 mL/min
0.25 mm ID (with EPC)	*	Pressure pulsing for 0.5 min. followed constant flow

* The EPC varies the head pressure to keep the flow constant. As the oven temperature rises, the head pressure increases to keep the flow constant. Without EPC, the head pressure stays constant and the flow decreases as the run progresses. The resulting flow listed is the average linear velocity (obtained at about 150°C). Pressure pulsing usually provides good results, with initial head pressures of 15 psi for 0.5 minutes and then a constant flow of 1.0-1.2 mL/min depending on your column and MSD.

Purge On Time: This is the time elapsed after injection that the system cycles from splitless (which is where it is during injection) to split (which is where it is for the balance of the run). I use a 0.50 minute time, but you should experiment +- 0.30 minutes to see what works best on your system.

to 69. By optimizing our tune parameters, we minimize the drain on the EM. This aids in stability and extends the multiplier life.

Here's what I recommend: Depending on your calibration range and data system, you should monitor the area of the first and last Internal Standards to see what works best. For example, say your first Internal Standard is 1,4-Dichlorobenzene-d4 its concentration is 40 ng/uL. Your quantitating ion is m/z=152. You run a curve and it looks great. You notice that the area of 1,4-Dichlorobenzene-d4 is 400,000 counts. Record this somewhere- you now know that all other things being equal, this is the sensitivity you need to obtain. Adjust your EMV as needed to keep the areas constant. The less they drift from day to day, the better your chance of staying linear. I go so far as to recommend to clients to record the areas of ISTD #1 and ISTD #6 in the run log for just this purpose. All systems are different, but you need to develop a feel for the area counts that work best for your system.

Another key to linearity is to insure proper split/splitless conditions. Each time you disassemble the injection port it should be leak checked. At the very least, check the total flow and head pressure; any changes from day to day indicate a leak.

Optimize the chromatography. In many situations, labs run with flows which aren't optimum. Many analysts believe low flows give better separation and chromatography. This is not always true. Better peak shape translates into better linearity and higher reproducibility. Remember, the mass spec sees ions, so selected ion profile peak shape counts, not total ion peak shape! Head pressures around 10-15 psi for 0.25 mm ID columns is a good guideline. If you have EPC, keep a constant flow of about 1.0-1.5 mL/min.

Certain compounds on EPA Methods 6255/8270/ TCLP can be difficult to obtain good linearity. You may run into the following problem compounds:

Benzoic Acid

This compound has several problems:

Problem	Solution
Poor peak shape	Thicker filmed columns improve peak shape; use ion 105 for quantitation.
Light sensitive	Store in amber vials or bottles.
Active	Keep injection port and first 3 meters of column clean. Cleanout weldment if response drops off.

Hexachlorocyclopentadiene

This compound has several problems:

Problem	Solution
Fragmentation pattern	Since it creates a cluster of ions (235, 237, 239), make sure peak shape and peak width's OK in Manual Tune. Use ion 237 as quant ion.
Light sensitive	Store in amber vials or bottles.
Active	Keep injection port and first 3 meters of column clean. Cleanout weldment if response drops off.

High boilers (i.e. the 6th Internal Standard plus the PAH's that quantitate off of it, especially the last three). Since these compounds are subject to poor vaporization in a cool injector, keep injection port at 250°C and insulated. Other problems which contribute to poor response: dirty inlet seal, clogged column, column not far enough into injection port (it should be about 4-5 mm), head pressure too low.

The Nitroaniline isomers. All three can be tough but 4-Nitroaniline is the worst:

Problem	Solution
Breaks down in hot injector	Keep injection port at 250°C
Poor peak shape	Quick to exhibit poor peak shape if the column is contaminated or worn out. Replace as needed.
Active	Keep injection port and first 3 meters of column clean. Cleanout weldment if response drops off.

Phenolics (mainly 2,4-Dinitrophenol, Pentachlorophenol, 4-Nitrophenol and 4,6-Dinitro-2 methylphenol): Various problems.

Problem	Solution
A. Fragmentation pattern	Keep mid-mass ions (131 and 219) in Manual Tune as high as possible while still passing DFTPP. Shoot for as close to 40% of each as possible.
Poor peak shape	Quick to exhibit poor peak shape if the column is contaminated or worn out. Replace as needed.
Active	Keep injection port and first 3 meters of column clean. Make

sure silanizing done correctly. Use fused silica wool as opposed to glass wool.

2,4,6-Tribromophenol.

Main problem is its high quantitating ion (m/z 330). If the mass axis gets skewed, it will create ion 329.65 and can be missed. Do axis calibration daily in Manual Tune and/or set the system to 329.7 instead of 330.0 in your compound list. If it persists in skewing the mass axis, a hardware problem probably exists.

Benzidine and 3,3'-Dichlorobenzidine.

Two main problems:

Problem	Solution
Light sensitive	Store in amber vials or bottles.
Active	Keep injection port and first 3 meters of column clean. Flush out lines in weldment.

EM Voltage. Since we are running capillary direct, it should be the same as what you used in Manual Tune. As stated previously, 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 good results!

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 scan range combined with 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. The scan range is determined by the method; the A/D mainly by the column size. For narrow bore (0.25 mm ID) columns, I recommend starting at 2^2=4, although ultimately you should choose the A/D setting which gives you best chromatography and overall results. A good guideline is to get about 10-12 scans across each peak.

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. A good exercise is to run a low level standard (let's say 5 ng). Do a spectrum scan at the apex of those analytes whose secondary or tertiary ions are at low percentages (i.e. <10% of the base peak). Obtain a tabulation of the abundances. If your settings are correct, your threshold will be approximately 1/2 the abundance of the qualifying ions at your low level.

Keys to Optimizing the Quantitation Routine

For you users with Enviroquant here are my tips on optimizing the Compound List:

- Retention Time Window: I recommend 1.0 minutes for Semivolatiles. That's ample time for the integrator to properly integrate. For Benzoic acid I recommend 2 minutes.
- Integration parameters: Use 5000 area counts for most compounds. Start threshold = .200; stop threshold 0.000. Data point sampling = 1. Smoothing box checked. Detection filtering =5 point. Don't forget to set your MDL cutoff in "Edit Quant Report Options".
- Curve fit: Average of response factors.
- Subtraction method: Use no abundance subtraction. I've found that the other methods are needed only if poor chromatography exists.
- 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.
- 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's better to widen the window and delete a mishit than to have too narrow a window and miss a compound that is present!
- 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. (NOTE: Re-tuning daily keeps required changes to a minimum).
- Update RT's and Q values daily. You can do this in "Update Levels".

- Use as many qualifier ions as possible. If the compound produces 4 qualifier ions that have >20% response, then enter them all.

- 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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