New Approaches to the Development of GC/MS Selected Ion Monitoring Acquisition and Quantitation Methods

Technique/Technology

Gas Chromatography/Mass Spectrometry

Author

Harry Prest
1601 California Avenue
Palo Alto, CA 94304
USA

David W. Peterson
1601 California Avenue
Palo Alto, CA 94304
USA

Introduction

Gas chromatographic analysis with mass spectrometric detection has become a routine analytical tool. The increased sensitivity of the new Agilent 5973N mass selective detector provides low detection limits with confirmation and quantitation of analytes in a variety of matrices.

Selected ion monitoring increases sensitivity for target analytes through the selective detection of ions most indicative of the compounds of interest. Gas chromatography/mass spectrometry-selected ion monitoring methods are useful and widely applied although they are somewhat complicated to develop and maintain, especially when the compound list is long. Recent enhancements in Agilent Mass Selective Detector Productivity ChemStation software (G1701- revision DA), such as the AutoSIM setup feature, have greatly reduced this problem.

Gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM) methods further benefit from retention-time locking [1,2]. Retention-time locking reduces the difficulty in maintaining selected ion monitoring acquisition and quantitation databases by making retention times permanent despite column maintenance or even replacement.

- A quantitation database for a retention-time locked method does not require updating after column maintenance or replacement; no laborious updating of compound retention times or SIM group windows is needed.

- GC/MS-SIM method with retention-time locking can be exported to any similar 6890 gas chromatograph/5973N mass selective detector instrument using the same column, next door or across the planet.

This note details a comprehensive approach to developing GC/MS-SIM methods using the retention-time locking and AutoSIM setup features of the Agilent 6890 gas chromatography, 5973N mass selective detector and ChemStation software.

Overview

The algorithm for developing GC/MS-SIM methods is:

1. Develop an appropriate gas chromatographic (GC) separation method.

2. Lock the compound retention times for the GC method.

3. Create a mass spectrometric (MS) quantitation database.

4. Generate the GC/MS-SIM acquisition method.

Agilent Technologies

Develop an Appropriate GC Method

Although it is likely the user has a preliminary approach to their method, GC technology is constantly evolving. It is worth considering whether using a different column, oven temperature or pressure program, etc., would improve the quality or efficiency of an analysis. We recommend consulting the Chemical Analysis section of the Agilent website (www.agilent.com/chem) under GC and gas chromatography/mass spectrometry (GC/MS) systems for a GC column chromatogram library and other references. Users should consult the Agilent website and consider attending one of the electronic-seminars offered by Agilent describing GC method development and the latest approaches (www.chem.agilent.com/cag/cabu/e-seminar.htm).

An appropriate GC method for selected ion monitoring (SIM) generates enough separation between compounds that there will be a minimum number of compounds in each SIM group. This will be addressed in more detail later in the note.

Lock the Compound Retention Times for the GC Method

The Mass Selective Detector (MSD) Productivity ChemStation software greatly simplifies retention time locking (RTL) setup and guides the user through the entire procedure. Refer to the ChemStation Help section or reference [3]. Three things are needed for successful RTL:

- A GC injection and oven program that provides the best separation results
- A compound that elutes in the middle of the oven program and can serve as a "locking compound"
- The retention time of the locking compound

Good choices of locking compound are internal standards that are present in every sample at a relatively high concentration. (For example, d_{12}-phenanthrene that elutes in the middle of the oven run for the polynuclear aromatic hydrocarbons (PAHs) and is relatively stable.) The locked retention time for this compound should be chosen to be within the extremes automatically produced by the RTL survey algorithm. This is easily discerned and decided by inspecting the data presented in the RTL setup.

Create an MS Quantitation Database

Acquire MS Data in Full Scan from a Standard

Using the retention time-locked GC method, make a full scan acquisition of the highest concentration standard containing all the compounds of interest that will be used in quantitation. (For good ion statistics, a "high standard" should deliver to the column greater than approximately 500 pg and preferably 1 to 5 ng.) The MSD should be tuned and scan parameters set such that the sampling rate is 2 (that is, 2^2).

Identify the Compound Ions and Set Up the Quantitation Database

Identify the compounds in the chromatogram and their retention times. It helps to make a labeled chromatogram at this point. There are two ways to set up a new quantitation database; manually and semi-automatically. Manual setup means that the compounds and their identifying ions are entered into the calibration table using the mouse. The minimum number of the most intense and unique ions for each compound should be chosen. Internal standards (ISTDs) must be placed in the table preceding the compounds to which they apply. This is where the labeled chromatogram and mass spectra for each analyte are helpful.

A semi-automated approach is also available in the CA revision and higher of the ChemStation software. AutoQuant searches an integrated chromatogram and sequentially identifies each peak by a library search of the mass spectrum (Data Analysis View/Calibrate/AutoQuant Setup). Be sure that the proper library is selected before executing AutoQuant and note that the sequence of entering the compounds cannot be controlled. The user is offered the choice of accepting the peak identification from the library search or specifying another name. The four most intense ions are automatically chosen and assigned as the quantitation ions in the order of their intensity.

Generate the GC/MS-SIM Acquisition Method

At this point, to set up the GC/MS-SIM acquisition method, the user must have:

- A retention time-locked GC acquisition method
- A quantitation database that contains the ions and retention times for all the compounds
- A full scan acquisition of the highest concentration standard that corresponds to and contains all the compounds in the database

In the Enhanced Data Analysis View, load the Method and the data file of the standard.

1. Even though there are no calibration levels in the quantitation database, select Quantitate / Calculate. This produces a report that lists the compounds and their retention times. Use this report to check that all the compounds have been found.

2. Use QEdit (under View/QEdit Quant Result) to check the integrations of the compounds. This is important for establishing the SIM windows so be sure the peak integrations are accurate. Correct the peak integration starting and ending points so peaks do not tail off excessively nor start too far in advance of the actual ion signal. Use QEdit to delete any peaks that are not of interest (impurities, column bleed peaks, etc.).

3. With the GC acquisition and database method loaded and the properly integrated data file of the high standard, execute the Method/Generate AutoSIM Method command. You will see a panel with parameters for setting up the SIM method. You will see a panel with parameters for setting up the SIM method, which is shown in Figure 1. These parameters control the SIM group setup and some are illustrated in Figure 2.
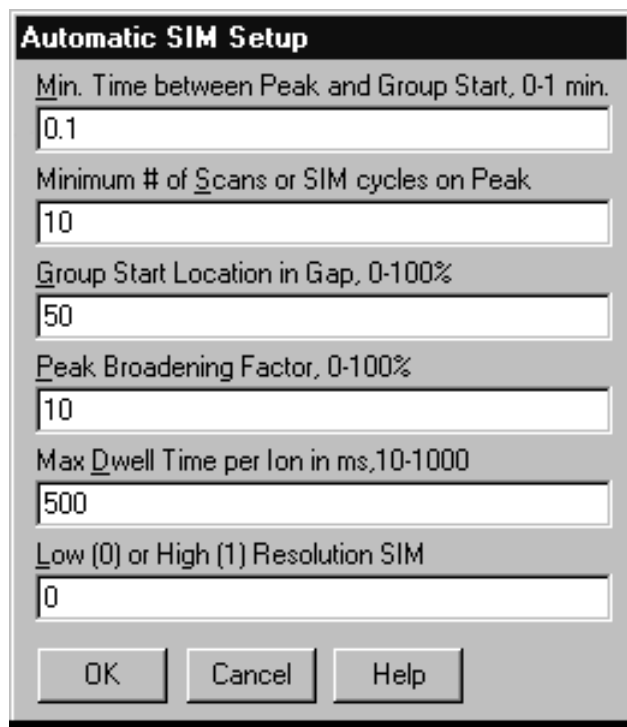

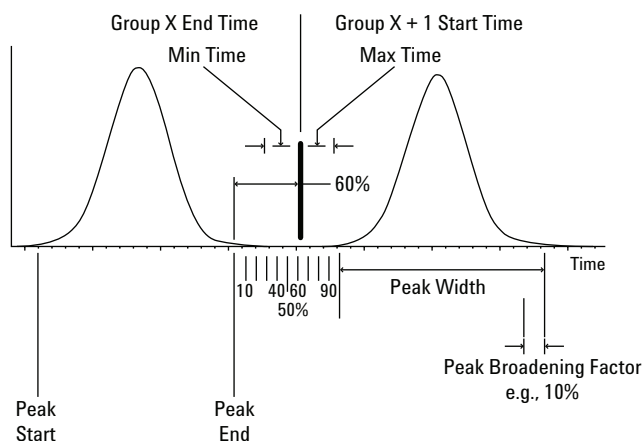Figure 1. AutoSIM setup parameter panel shown with the default setup parameters.

Figure 2. Illustration of the SIM parameters.

SIM Setup Parameters

Minimum Time Between Peak and Group

This parameter sets the minimum time between a group start and either the end of or the beginning of a peak. The default is 0.1 min (6 secs). This parameter has the most influence on the number of SIM groups. Lowering the time creates more groups.

Minimum Number of Scans or SIM Cycles on a Peak

To obtain good quantitation, the number of scans across an analyte peak should be on the order of 10 scans (the default). Increasing this parameter will decrease the ion dwell times in each group and give more scans across analyte peaks.

Group Start Location in Gap, 0-100%

This parameter controls when a group starts in the gap between peaks. The user can control how far a group start position is from the tailing edge of the last peak in the previous group, or how close the group start is to the first peak in the new group. The default of 50% places group start times exactly in the middle between the end of the last peak in the previous group and the first peak in the new group. Increasing this value moves the group start times later or further from the last peak. If peaks tend to tail, increase this value. For example, Figure 2 illustrates a value of 60% of the gap between peaks.

Peak Broadening Factor, 0-100%

A particular peak may tail or show poor peak shape. This factor adds a "safety margin" for broader peaks as a percentage of their peak width. Start with this parameter at the default of 10%. If the chromatography produces very sharp peaks, set this to 0%.

Maximum Dwell Time per Ion in ms, 10-1000

This parameter sets the maximum allowable dwell time for ions in a group. This overrides the calculated ion dwell time based on achieving the specified number of scans over a peak when the calculated value exceeds this maximum. The default is 500 milliseconds.

Low (0) or High (1) Resolution SIM

Because this algorithm is based on scan results for the ion m/z ratios, low resolution is the recommended default. If the user wishes to set up a high resolution SIM method, the ion m/z values should be considered tentative assignments (since they were acquired by scan) and subject to optimization by changes in 0.05 m/z increments around these initial values. If this method is moved between instruments, ion m/z values should be checked. Tuning will bring the instruments to an agreement of 0.10 to 0.15 amu but SIM will allow specificity to 0.05 amu.

After selecting the parameters and selecting OK, the algorithm will choose the SIM group times, place the compound ions in each of the groups and assign dwell times. The software will then offer the option of printing a report. The report consists of:

- A graphic for both the merged and the total ion chromatograms labeled with analyte peak numbers from the quantitation database and SIM group numbers

- *A SIM Group Switching Report* which gives the group start times, the number of compounds in the group, the number of ions and their dwell times and other parameters

- *A SIM Ion Report* which shows which compounds are in which SIM groups and why. The 'Cases' for why a compound has been assigned to a group are very helpful for improving the setup. An example is given below in the Addendum section *SIM Setup Advice.*

At this point, if the objectives of the analyst are met, the SIM groups can be saved to the method and the method should be saved. The method should then be applied to the highest standard to confirm that all the compounds are acquired in SIM and that the SIM windows are correct. If everything checks out, all the standards can be run and the compound responses added to the quantitation database.

Conclusions

There are four steps to sensitive analysis by GC/MS-SIM; selecting a GC column and developing a method, applying retention-time locking to the method, building a quantitation database with entries for each of the target compounds and applying the AutoSIM macro. Agilent's GC Columns and Supplies group has extensive knowledge on GC columns and methods for a wide range of applications and should be considered a resource for information. RTL enables the user to create permanent methods for sensitive analysis of target compounds. RTL setup is automated, easy to implement and has consistently improved productivity in customer laboratories.

The convenient setup and maintenance of Quantitation Databases and SIM methods is a key feature of the ChemStation Software. These features of the DA version of the ChemStation Software are a powerful combination for creating robust GC/MS methodologies and will find widespread application across all markets.

Addendum: SIM Setup Advice

Fine Points and Philosophy

The AutoSIM algorithm is very rugged. Complications will occur in cases where the analyte list is very complex with many compounds which coelute or are minimally separated. Remember, there is no substitute for good chromatography. The constraints on SIM are that there cannot be more than 50 groups and that each group can have a maximum of 30 ions. At a maximum of 4 ions per compound, no more than seven or eight compounds can be accommodated in a group or, in theory, a total of 350 to 500 compounds in a method.

To get the best quantitation results, there should be 8 to 12 cycles over a peak. The minimum dwell time is 10 msec so peak widths must exceed 3 seconds for a group of 30 ions. The objective is to have the fewest number of compounds in a group, which means use as many groups as possible and choose the minimum number of identifying ions for each compound. If the chromatography has been optimized and still too many compounds are in a group, take advantage of the highly precise retention times available in the 6890 GC and lower the "Minimum Time between Peak and Group Start". This will increase the number of SIM groups. Also set the 'Group Start Location in Gap' to 50%.

If there are too many ions in a group, the software will warn the user on the screen and flag the group(s) in the *SIM Group Switching Report*. The user should then look at those groups that fail, recheck the integrations of the peaks and then decide whether to:

• Improve the chromatography in that region

• Use fewer ions for compounds for some or all of the affected compounds

• Change the setup parameters; setting the Group Start Location in the Gap to 50%; lowering the percentage of peak broadening to 0%; shortening the 'Minimum Time Between a Peak and a Group Start' parameter in small (0.01 min) increments

For example, Figure 3 shows a GC/MS total ion chromatogram for 83 compounds that are part of the EPA 8270 method. Notice the separation is very poor in the 6 to 13 minute region.

Applying the defaults of the SIM setup produces 13 SIM groups and a warning that there are too many compounds for use of all the quantitation ions in the 3rd and 4th groups (Figure 4). Reviewing the SIM Ion Report shows there is not enough time between peaks to start a group ("Case 2" situations). This means looking for the largest gap between the end of one peak and the beginning of another will guide setting a shorter "Minimum Time Between a Peak and a Group Start" parameter. There are two approaches; review the gaps and find the largest gap and divide by two to find a new time parameter or the easier approach of iteratively shortening the minimum time by 0.01 increments.

Shortening the "Minimum Time Between Peak and Group Start" parameter increases the number of groups and eventually generates a method which allows for all the ions. Notice this has 19 SIM groups as shown in Figure 5.
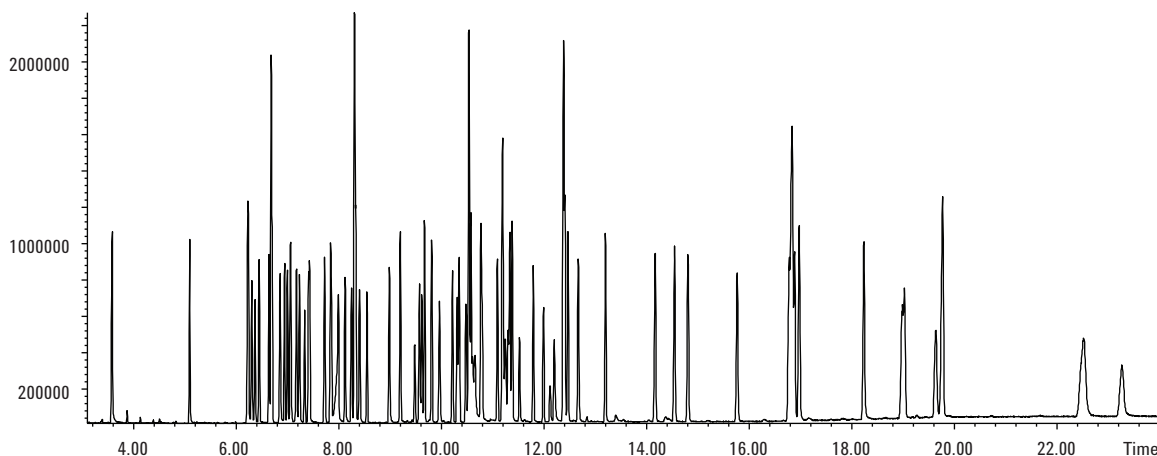

Figure 3. Total ion chromatogram of the full scan acquisition of the highest standard in a SIM method for 83 compounds as part of EPA Method 8270.
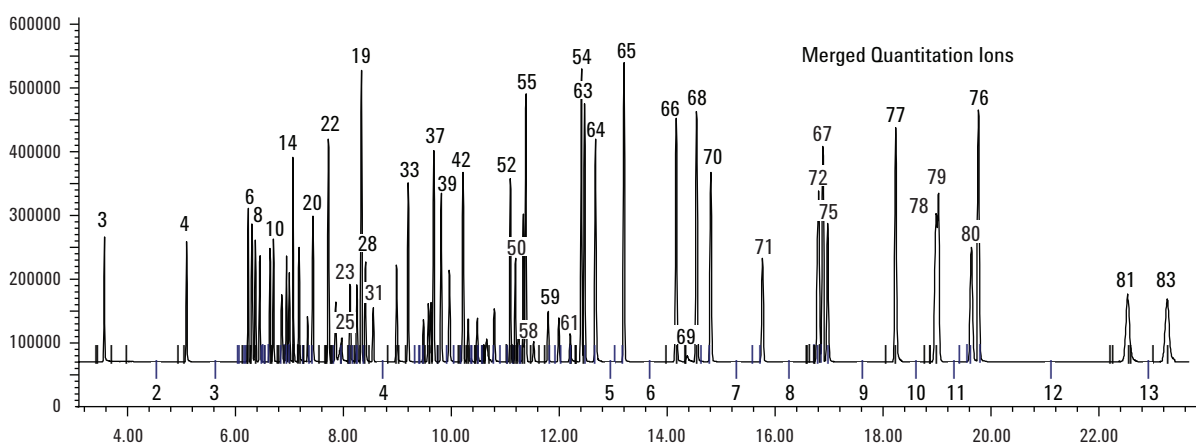

Figure 4. Merged quantitation ion chromatogram of the highest standard in a SIM method generated by the default SIM setup settings. The SIM group numbers of the 13 SIM groups are indicated by the numbers below the baseline. Compound numbers label the peaks. Notice Group 3 (starting near 6 minutes) and Group 4 (starting near 9 minutes) have roughly 30 compounds in them.
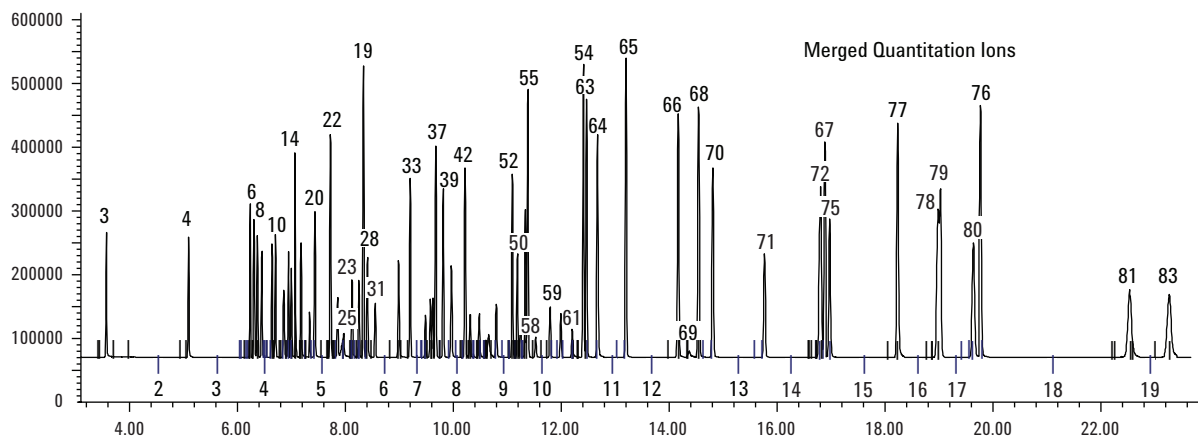
5

Figure 5. Merged quantitation ion chromatogram of the highest standard in a SIM method generated by the SIM setup settings with a shorter time for the "Minimum Time Between Peak and Group Start" parameter. The total number of SIM groups have gone up from 13 SIM groups to 19 to accommodate all the compounds.

References

1. Giarrocco, V., B. Quimby, and M. Klee, Retention Time Locking: Concepts and Applications, Agilent Technologies, 5966-2469E, www.agilent.com/chem

2. Prest, H. and P. Cormia, Retention Time Locking: Advantages in GC/MS SIM Analysis, Agilent Technologies, Application Brief (23) 5967-3797E, www.agilent.com/chem

3. Agnew, D., et al., Retention Time Locking with the G1701BA MSD Productivity ChemStation, Agilent Technologies, 5968-3433E, www.agilent.com/chem

For More Information

For more information on our products and services, visit our Web site at: www.agilent.com/chem.

Agilent Technologies