5973 Inert Performance Electronics: Considerations for GC/MS Methods in Scan and Selected-Ion Monitoring Modes

Application

Technique/Technology

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Abstract

Since higher productivity is the key to staying competitive, GC analyses have had to become more rapid. To contend with faster chromatography, faster mass spectrometric acquisition is required to better capture the information contained in the narrower peaks. In the past, scanning faster meant significant losses in signal, sensitivity, and spectral fidelity. The new Performance Electronics of the Agilent Technologies 5973 Inert MSD was developed to maintain signal intensity and spectral quality. These electronics also provide scan speeds up to 10,000 amu per second. This note describes aspects of these improvements important to the successful implementation of more rapid gas chromatography/mass spectrometry analysis, in both scan and selected-ion monitoring modes.

Introduction

In method development, the analyst chooses the column specifications (phase, film thickness, capacity), the oven program, etc. appropriate to the analytes, the range of concentrations of interest, sample related issues, and the desired runtime. In developing this separation method, chromatographic peaks of a specific width are generated for each compound. This peak width, as measured at the base in seconds, is the important chromatographic parameter as far as the mass spectrometer is concerned.

One of the rules of gas chromatography/mass spectrometry (GC/MS) acquisition, in both scan and selected-ion monitoring (SIM) modes, is to never acquire faster than necessary. Increasing scan speeds or decreasing ion dwell times always results in signal losses, in inferior spectral fidelity, and lower ion-ratio accuracy or precision. The question is always, “How fast is necessary?” The answer is always based on what needs to be established.

Scan Considerations

Scan acquisitions favor either qualitative surveys of samples for compounds or more quantitative studies. When focused on qualitative surveys, spectral quality is most important and detection of unknowns or unexpected compounds is a priority. For example, in screening samples for pesticide residues, full-scan spectral quality is important for compound detection. The more quantitative studies tend to have target compound lists and, although a subset of ions is typically used in quantitation, the full-scan compound spectra are required for confidence.

With regard to these quantitative studies, there are constraints imposed on ion-ratio accuracy and reproducibility. Early in the development of more commercial aspects of MS, an article appeared which examined scan functions and modeled scanning over a chromatographic peak to accurately establish ion ratios [1]. The authors demonstrated that 10 scans were necessary to establish ion ratios to approximately 1%. This has become the guidance for acquisitions in both scan and SIM modes. In practice, GC/MS parameters are arranged such that 8 to 10 scans are acquired over a peak to ensure a good mapping.

As regards the qualitative studies wherein spectral quality is paramount, 4 to 5 scans over a peak is sufficient. For example, the Agilent Deconvolution Reporting Software (DRS), that employs the NIST AMDIS searching algorithm, is capable of interpolating peak apexes and ion coincidence to within a quarter of a scan [2]. Scanning slowly with more averages over a peak provides superior spectra and less noise and therefore better opportunities for compound detection.

So in GC/MS scan method development, given a particular separation for a series of compounds (that is, established and known peak widths for all compounds of interest), a scan speed appropriate to the analyst's intentions can be selected. The last two parameters for the mass spectrometer are the mass range to be scanned and the number of samples, n, to be taken at each mass acquired. These two parameters determine the effective scan speed. The “effective” scan speed is not the same as the often-cited electronic scanning speed but is the actual speed to which data is acquired and written to the hard disk. In other words, the speed at which useful information is presented to the user in a data file.

The “number of samples”, n, is related to the number of times a particular mass is acquired before moving to the next mass (actually *m/z*). Essentially this is proportional to the number of averages taken at a particular mass. This is selectable in factors of 2 as 2^n where n has values 0, 1, 2, 3, 4, 5, 6, and 7 and sampling (averages) are 1, 2, 4, 8, 16, 32, 64, and 128. Less sampling, or lower n values, means more rapid cycling through the selected mass range, thereby achieving higher effective scan speeds. Conversely, more averages obtained with higher sampling and larger n values results in a lower effective scan speed for a given mass range. Table 1 illustrates the connection between sampling parameters and scan rate (electronic).

Table 1. Samples (n), Samples/Step, and Scan Speed

2^n	2^3	2^2	2^1	2^0	Fast Scan
Samples "n"	3	2	1	0	0
Samples/step	8	4	2	1	1
Electronic scan speed [amu/s]	781	1562	3125	6250	10000

Figures 1 and 2 display the influence of mass range and samples, n, versus the compound chromatographic peak width for quantitative and qualitative studies, respectively. To use or understand these plots, measure the peak widths for compounds at their base (PW_{base}), and calculate the mass range over which scanning is required. Read across from the peak width and up from the mass range to find the region of intersection. For example, consider an acquisition that produces peaks about 0.10 min or 6 seconds in (base) width. Figure 1 shows that at a sampling of n = 3 (2^3), 10 or more scans over the peak will be obtained up to a mass range of 450 *m/z*. Beyond a mass range of more than 560 *m/z*, fewer than 8 scans will be acquired over a 6-s peak. If the GC analysis is accelerated and the peak width narrows to 3 s and the same mass range is required (450 *m/z*) at 10 scans/peak, the speed must be increased by lowering the sampling to 2^2.

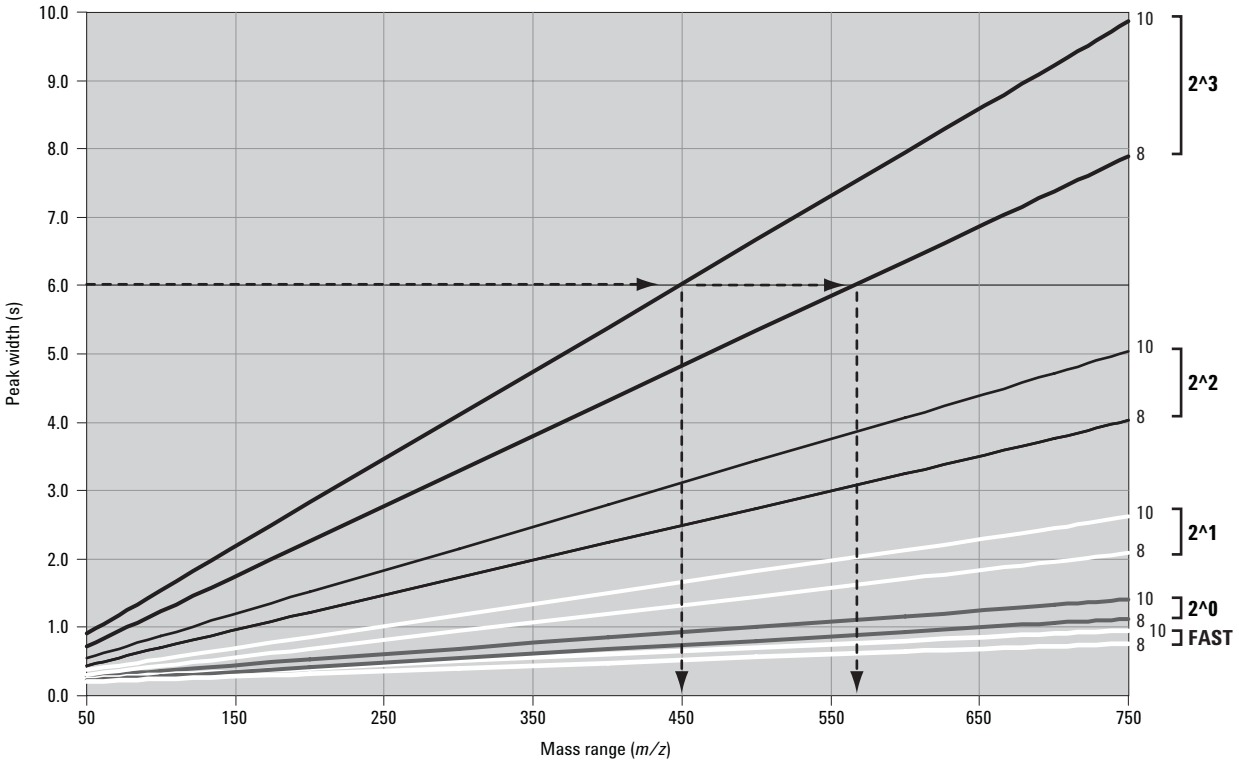

Figure 1. Plot of mass range (x-axis) versus permissible scan speeds to obtain the 8 to 10 scans required for quantitative GC/MS studies over a given chromatographic peak width (y-axis). The upper and lower lines for each sampling are labeled to indicate the number of scans obtained per peak width and range as 10 scans for the upper and 8 scans for the lower line. The dotted line illustrates the example given in the text for a 6-s chromatographic peak.

Another approach particularly helpful in designing methods, is given a mass range to be scanned, one can see what peaks widths are accessible by particular speeds. For example, given a mass range of 450 *m/z* :

PW_{base} > 5 s implies sampling at n = 3 and the speed 2^3

5 s > PW_{base} > 3 s sampling at n = 2 and the speed 2^2

3 s > PW_{base} > 1.5 s sampling at n = 1 and the speed 2^1, etc.

In design of MS methods for acquisitions where detection and spectra are most important, as in the qualitative assay scenario, scan parameters should be arranged to provide no less than 4 scans over a peak. The relationships between peak width, scan range and sampling to provide 4 to 5 scans over a peak are provided in Figure 2. This regime is appropriate to use with the new Agilent DRS that uses the NIST program AMDIS. The AMDIS program is capable of discerning compounds separated by a fraction of a scan. However, here four scans over peaks should be considered a lower limit and Figure 2 can be used to test acquisition designs.

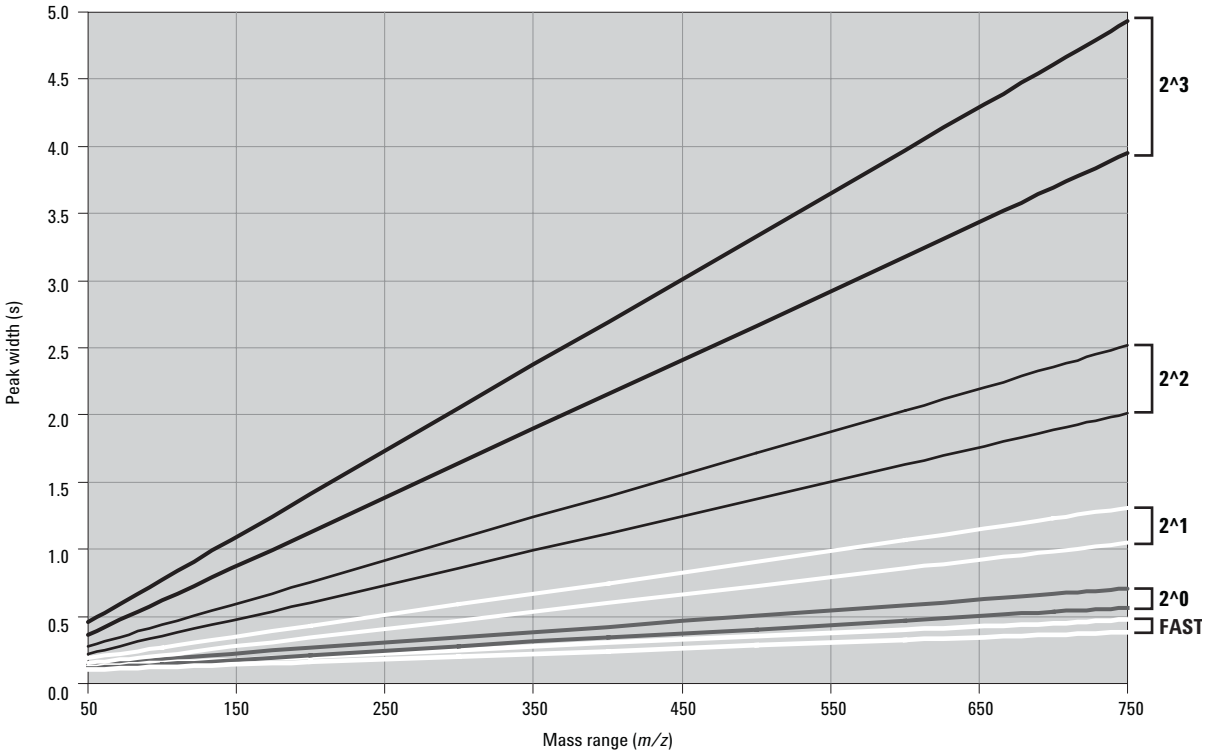

Figure 2. Plot of mass range (x-axis) versus permissible scan speeds to obtain the 4 to 5 scans recommended for qualitative GC/MS studies over a given chromatographic peak width (y-axis). The upper and lower lines for each sampling are labeled to indicate the number of scans obtained per peak width and range as 5 scans for the upper and 4 scans for the lower line.

Notice the slopes of the corresponding lines in Figure 2 are exactly half those of Figure 1. This simply shows that the speed requirements for a unit time of chromatographic peak width increase with increasing mass range and decreasing peak width.

Detailed Example

Experimental

A standard of 20 PCBs in isooctane was analyzed under the conditions given in Table 2. Scan speeds for the mass range of 150 to 510 *m/z* were selected in the MS parameters setup panel by changes in the number of samples from 3, 2, 1, and 0. Fast Scanning mode (10,000 amu/s) was also invoked (see Figure 3).

Table 2. GC and MSD Configuration and Parameters

Injection parameters			
Injection mode	Pulsed splitless		
Injection volume	1 µL		
Injection port temperature	275 °C		
Pulse pressure and time	25.0 psi		0.50 min
Purge flow and time	50.0 mL/min		1.00 min
Gas saver flow and time	20.0 mL/min		3.00 min
Column and oven parameters			
GC column	HP-5ms 30 m x 0.25 mm id, 0.25 µm film p/n: 19091S-433		
Flow and mode	1.3 mL/min		Constant flow
Detector and outlet pressure	MSD		Vacuum
Oven temperature program	50 °C		1.00 min
	45 °C/min	325 °C	1.60 min
Oven equilibrium time	1.0 min		
Total program time	8.71		
MSD transfer line temperature	325 °C		
Mass spectrometer parameters			
Tune parameters	Autotune		
Electron multiplier voltage	Autotune +400 V		
Solvent delay	4.50 min		
Scan parameters	150–510 *m/z*		
Threshold	150		
Sample number	3, 2, 1, 0, and Fast		
Quadrupole temperature	150 °C		
Source temperature	250 °C		
Miscellaneous parts			
Part	**p/n**		**Description**
Septa	5182-0739		BTO septa (400 °C)
Liner	5181-3315		Deactivated 4-mm id double taper
GC column ferrule	5181-3323		250 µm Vespel/Graphite
MSD interface ferrule	5062-3508		0.4-mm id, preconditioned Vespel/graphite

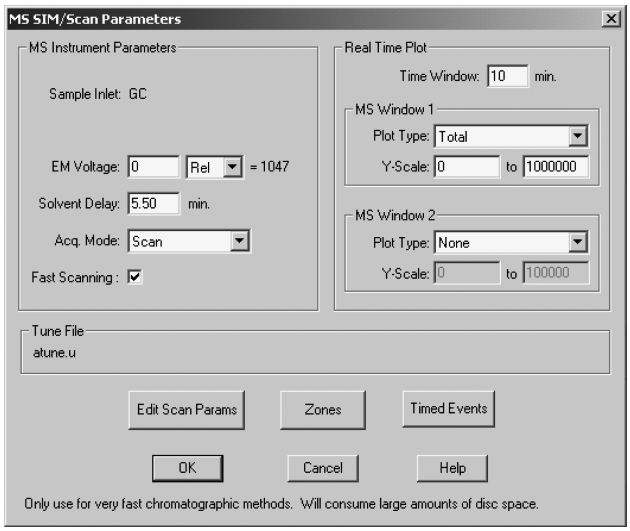

Figure 3. MS parameters setup for enabling Fast Scanning mode.

Figure 4 shows a rapid total ion chromatogram (RTIC) for 20 PCBs acquired in scan in under 8.5 minutes. The first peak, biphenyl has a peak width at the base of ~2.7 seconds.

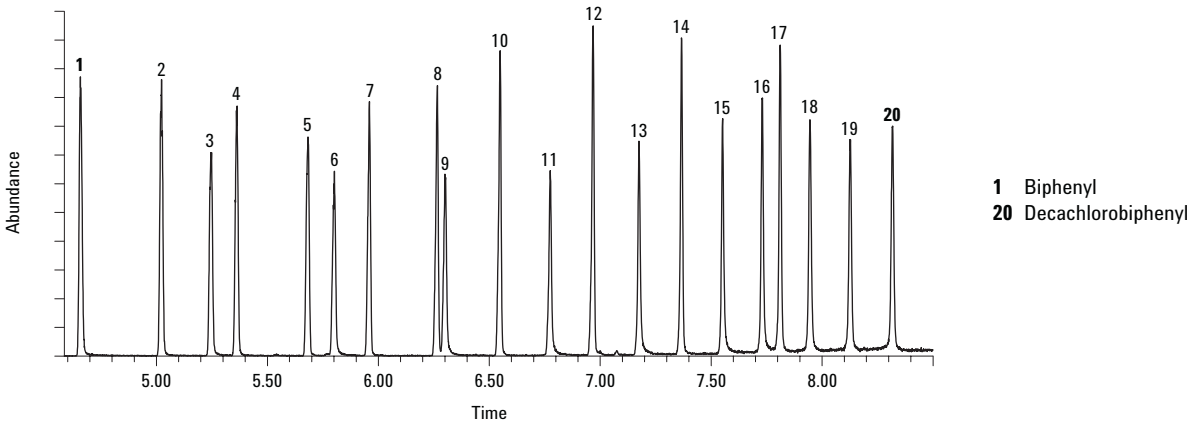

Figure 4. RTIC for rapid scan acquisition of 20 PCBs ranging from biphenyl to decachlorobiphenyl, including two representatives for each degree of chlorination, in full scan on an HP-5ms (30 m × 0.25 mm id × 0.25 µm) column. Peak widths at the base average about 3.9 s.

Figure 5 shows the biphenyl peak under all speeds available to the performance electronics of the Agilent 5973 Inert MSD and lists the approximate number of scans over the peak.

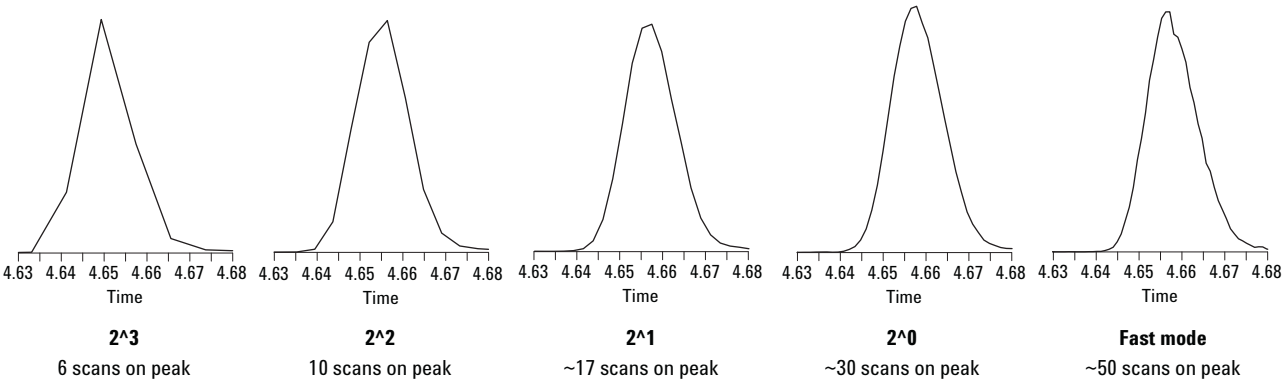

Figure 5. RTICs for the biphenyl peak, as acquired at each scan speed for the mass range 150–510 amu.

At the slowest speed, 2^3, there are about 6 scans over the peak. The plot of peak width versus scanned mass range for quantitative studies (Figure 1) suggests that a peak under 3 s and a scanned range just over 350 *m/z* will have less than eight scans at a sampling of 2^3. Similarly, Figure 2 predicts for these same parameters, that greater than 5 scans will be obtained. At the 2^2 sampling rate, Figure 5 shows about 10 scans across the peak - the same as the 10 predicted by Figure 1. The higher speeds presented in Figure 5 show approximately a doubling of the number of scans over each peak for each increment in speed or each halving of sampling. The data show the predictions valid and they suggest that 2^3 is sufficient for qualitative work and 2^2 is required for quantitative work.

An additional consideration is the other peaks in Figure 4. The last peak, decachlorobiphenyl, is slightly broader, as is typical for later eluting components. This broadening is less than ~0.5 s here and is not a concern; optimizing our scan settings for the biphenyl peak, the narrowest peak in the chromatogram, we are guaranteed to obtain sufficient scans. This case is true for most situations in constant flow mode but not true for constant pressure mode. In constant pressure mode, peaks may be significantly different in width from the beginning to the end of the chromatogram. In this case

we may be over-sampling the later peaks and increasing the compound detection limits unnecessarily if we consider only the earliest or narrowest peaks. Similarly mass ranges may be optimized based on the elution times. The MSD Productivity ChemStation SW (G1701DA) allows up to three scan segments to be configured to allow different mass ranges, thresholds and sampling to be applied over the course of an acquisition to address these changes.

Scan Considerations - Conclusions

The data in Figure 5 suggests the question, "why not go faster?" After all, if 10 scans are good, aren't 20 scans better? Why not run in Fast mode all the time? There is a price to be paid in scanning faster. Not only is going too fast unproductive, as suggested in reference 1, but the price is a loss in response and a decrease in spectral quality. The new Performance Electronics of the 5973 Inert MSD vastly improves this situation, and increasing scan speed does not show the large loss in signal as previously experienced. Going faster means less sampling (that is, fewer averages taken at a mass) which means that the spectra become "noisier", and is unavoidable. The loss in response is a function of tuning and compound fragmentation character, so generalizations are difficult. However, the new Performance Electronics do maintain accuracy in mass assignments even at the highest speeds, and users can expect this to be <0.3 amu (which is half the typical AutoTune peak width).

In summary, the following guidance is given:

- Scan as short a mass range as possible.
- Scan as slowly as possible to obtain a sufficient number of scans over a chromatographic peak, which is 10 scans for quantitative applications, and no less than 4 scans for qualitative applications.

SIM Considerations

In many ways the situation in SIM is similar to that in scan, however, because SIM tends to be applied in target compound analysis, SIM methods typically are designed for quantitation. In this respect, SIM methods require 10 scans over the peak to be accurate to the %RSD level. As chromatography becomes more rapid there are two possible effects: crowding the chromatographic space and contraction of the peak width.

Figure 6 is a plot similar to that of Figure 1.

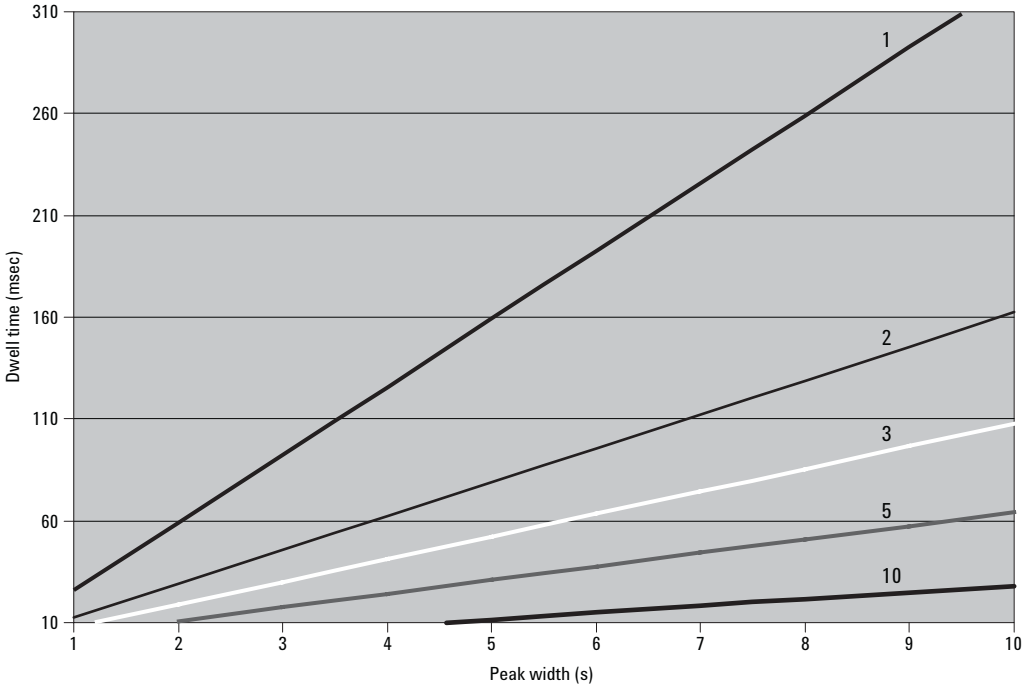

Figure 6. Plot of peak width (s) versus the ion dwell time required to produce 10 scans. The lines are based on three ions per compound and also the number of compounds in a group. Therefore, the line labeled as 1 is one compound with three SIM ions in the group; the line labeled as 2 represents two compounds in the group, both with three ions, so six SIM ions per group; etc., up to 10 compounds with three ions or 30 ions in the SIM group.

This plot gives individual ion dwell time in a group versus the peak width as required to produce 10 scans over a peak. The plot is based on three ions per compound and shows the affect of increasing the number of compounds in an ion group and decreasing the peak width. Simply put, as the chromatography becomes faster and peaks sharper, dwell time decreases. And as peak widths narrow, the number of compounds allowed in a group decreases. This is because there are limits to the number of ions allowed in each group (30), the number of groups (50), and the chromatographic space available. Another limit is the minimum ion dwell time of 10 ms.

SIM methods suffer from two difficulties - method setup and method maintenance. These problems are solved by AutoSIM and Retention-Time Locking (RTL) which are described in detail elsewhere [3]. Briefly, RTL makes compound retention times permanent so they do not change after column cutbacks or column replacement. This means SIM group times do not need constant upkeep but can be made immutable. Based on a full-scan acquisition of a standard, the AutoSIM software macro automatically parses the chromatogram to assign compounds to groups, assign the compound ions to each group, and calculates and sets the dwell times for the ions in the group. The user can assign the number of scans to be acquired over the peak - this is usually set to 10. The new Performance Electronics allow more rapid acquisitions and AutoSIM may under-estimate the ion dwell times and produce more than the requested scans over the peak. Simply back off the number in the AutoSIM setup and recompile the method.

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Existing methods need not be altered but will produce more scans over the peaks. The increase will be most pronounced for groups with many ions and short dwell times. This may allow the user to accelerate their method to take advantage of the more rapid SIM available in the Performance Electronics.

References

1. D.E. Matthews and J.M. Hayes, “Systematic Errors in Gas Chromatography-Mass Spectrometry Isotope Ratio Measurements”, (1976) *Analytical Chemistry*, **48**, 1375-1382.
2. P.L. Wylie, et al, “Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software”, Agilent Technologies, publication 5989-1157EN, www.agilent.com/chem
3. H. Prest and D.W. Peterson, “New approaches to the development of GC-MS selected ion monitoring acquisition and quantitation methods.” Agilent Technologies, publication 5988-4188EN, www.agilent.com/chem

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