The 5975C Series MSDs: Method Optimization and Trace Ion Detection

Technical Overview

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Introduction

The new software control offered by the ChemStation Software (G1701EA) and hardware improvements of the 5975C MSD give the user several avenues for method improvements. They should be considered in this order.

Higher Ion Source Temperatures [1]

Ion source operating temperatures as high as 350 °C are possible in the 5975C MSD. By upgrade, 5975A&B and 5973 MSD systems can do the same. Source temperatures higher than the 230 °C default can provide improvements in compound response, chromatographic peak shape, and longevity of operation.

Gain Normalized Tuning [2]

Tuning the MSD to target a specific electron multiplier (EM) gain instead of depending on the normal approach of tuning to abundances can be of enormous benefit. Better and more consistent compound responses even as the multiplier "ages" can be obtained as well as more comparable results between different instruments. Greater flexibility exists by targeting specific EM gain. For relatively high-concentration samples, a lower gain will allow an optimum and reproducible operating range. For low concentrations and trace analysis, the HiSense.U tune sets an EM gain of 15×10^5 and will provide reproducible detection and quantitation for operating near the method detection limits.

Trace Ion Detection

One of the most important contributions that computerized data systems and advanced electronics have provided mass spectroscopy is the ability to process the raw acquired data. These routines have been operating in the background either in firmware or in post-processing for many decades and continue to be a rich avenue of research. Essentially these algorithms process the raw data to generate peak mass and abundance assignments so that when users look at a spectrum or the reconstructed ion chromatograms, they see a coherent and simplified rendering of what the instrument measures. With the advent of the Performance Electronics package, Agilent began to examine possible improvements in the existing approach to peak detection, keeping in mind our users' broad range of applications but with specific attention to compound detection in trace analysis—the most challenging area. This lead to the development of the Trace Ion Detection (TID) mode of operation available in the ChemStation G1701EA software of the 5975C MSD.

Enabling Trace Ion Detection

In the Chemstation G1701EA software, TID is enabled under the Instrument menu (see Figure 1). Simply setting the check box and saving the method creates an acquisition method that will acquire with TID operating.

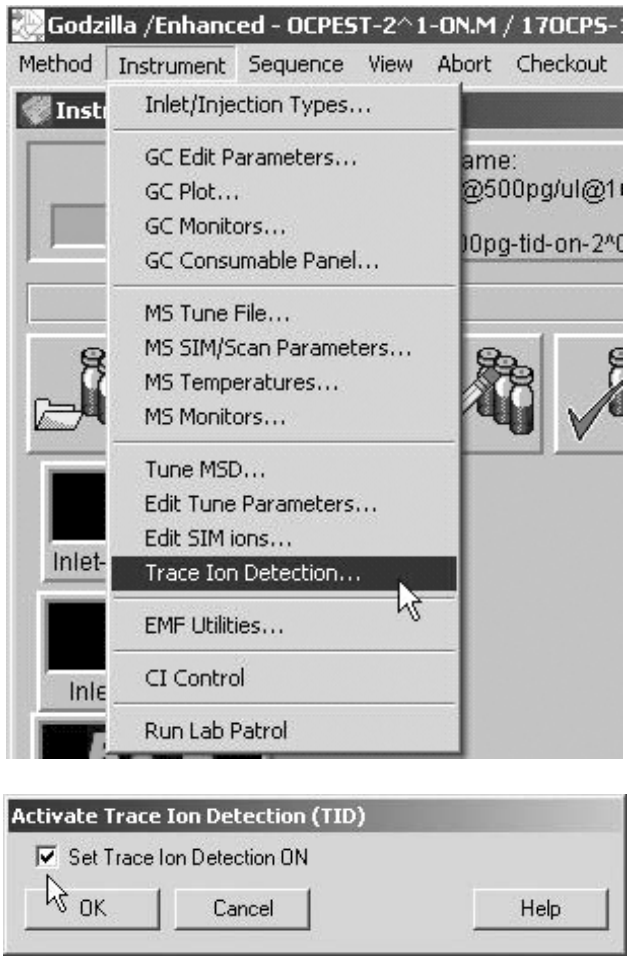

Figure 1. Enabling trace ion detection.

It is critical that compound calibration curves be composed of data consistently acquired either with TID on or TID off and not some mixture. TID will effect peak heights and areas and therefore compound responses.

TID Considerations and Application

There has always been a trade-off in designing mass spectrometric methods. The rule "scan as slowly as possible" has always required careful consideration in choosing the scanning speed of the instrument. Faster scan speeds give more data points across the chromatographic peak[1] but the faster speeds lower the ion statistics and make peaks and spectra appear "noisy." Lower scan speeds produce fewer scans over the peak but better spectra and so better library search results, ion ratios, etc., and so the compromise. Mass spectrometrists have always used eight or more scans per peak as a rule of thumb for obtaining good quantitation results and more than four scans per peak for good library hits. They have also recognized that scanning faster than necessary, as determined by the compound chromatographic peak widths, results in "noisier" data and degraded ion statistics and consequently degraded detection limits. To ameliorate these effects many have looked toward improved situations in hardware and software. The Performance Electronics package of the 5973 Inert and 5975 Series MSDs represents such an improvement. Software approaches like advanced peak detection algorithms have been widely explored but Trace Ion Detection represents a unique technical innovation.

In a certain sense TID is a "filtering" algorithm so peaks will look "smoothed." This filtering is an advanced form of "averaging" so there are implications for peak shape that must be expected, specifically a loss in peak height and some peak broadening. The degree of change is directly reflected in the number of samples over the peak. Consider as an example the reconstructed extracted ion chromatograms (REICs) for the quantitation ions of four compounds shown in Figure 2. The panels show that as the number of scans over the peak increases, the TID ON and TID OFF chromatographic peak heights become more similar and the peak shapes are more closely matched. In all cases the peak areas agree to within 10% so there is no loss. This is because although the peak height decreases with TID, the "background" also decreases and the peak slightly broadens to produce nearly the same areas.

TID and Compound Spectra

At higher concentrations, as shown in Figure 2, the general effect of TID can be seen; however, its effectiveness is only apparent at lower concentrations where noise begins to influence the signal. A first consideration would be the effect of TID on spectral quality. Figure 3 shows the results of a PBM search against the NIST 2005 spectral library for four compounds at low concentrations in a standard acquired with and without TID. The peaks are labeled with the PBM match quality and show a significant improvement in match quality when applying TID. Note in the right-hand side panels the improved spectra, which can be seen in the mass range below 200 *m/z* where the absolute intensities of the intermediate masses are suppressed. Overall, the reconstructed total ion chromatogram's baseline in the TID mode shows less variance than without TID as a reflection of the noise reduction.

[1] In most cases, the term "peak" will refer to the chromatographic peak generated by an ion current or the total ion current and belonging to a compound and not features within a mass spectrum.

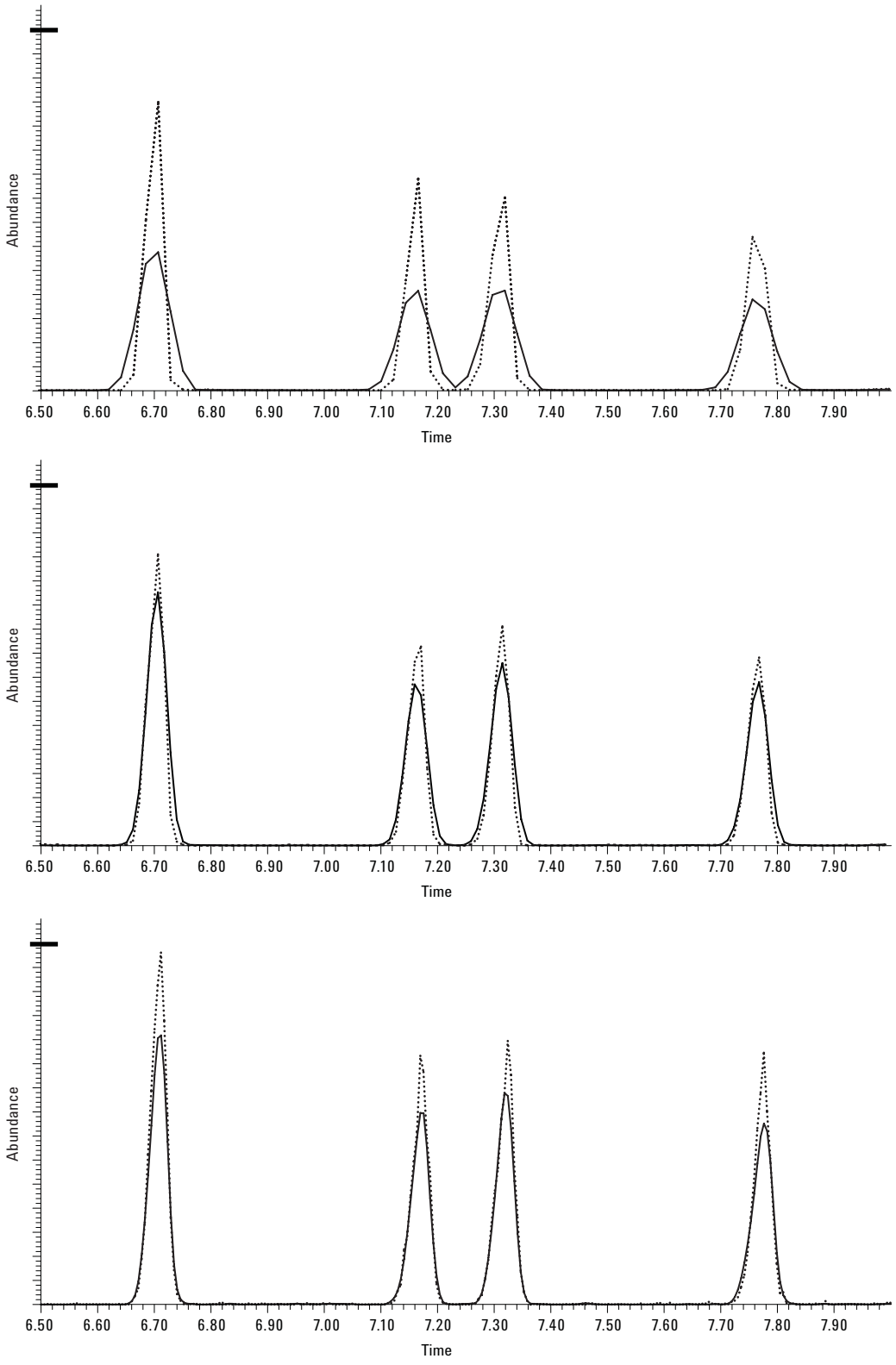

Figure 2. Trace Ion Detection mode ON (——) versus OFF (·····). REICs of quantitation ions showing the effects on chromatographic peaks acquired with different samplings: upper panel, 5 scans/peak; middle panel, 9 scans/peak; lower panel, 18 samples over the peak.

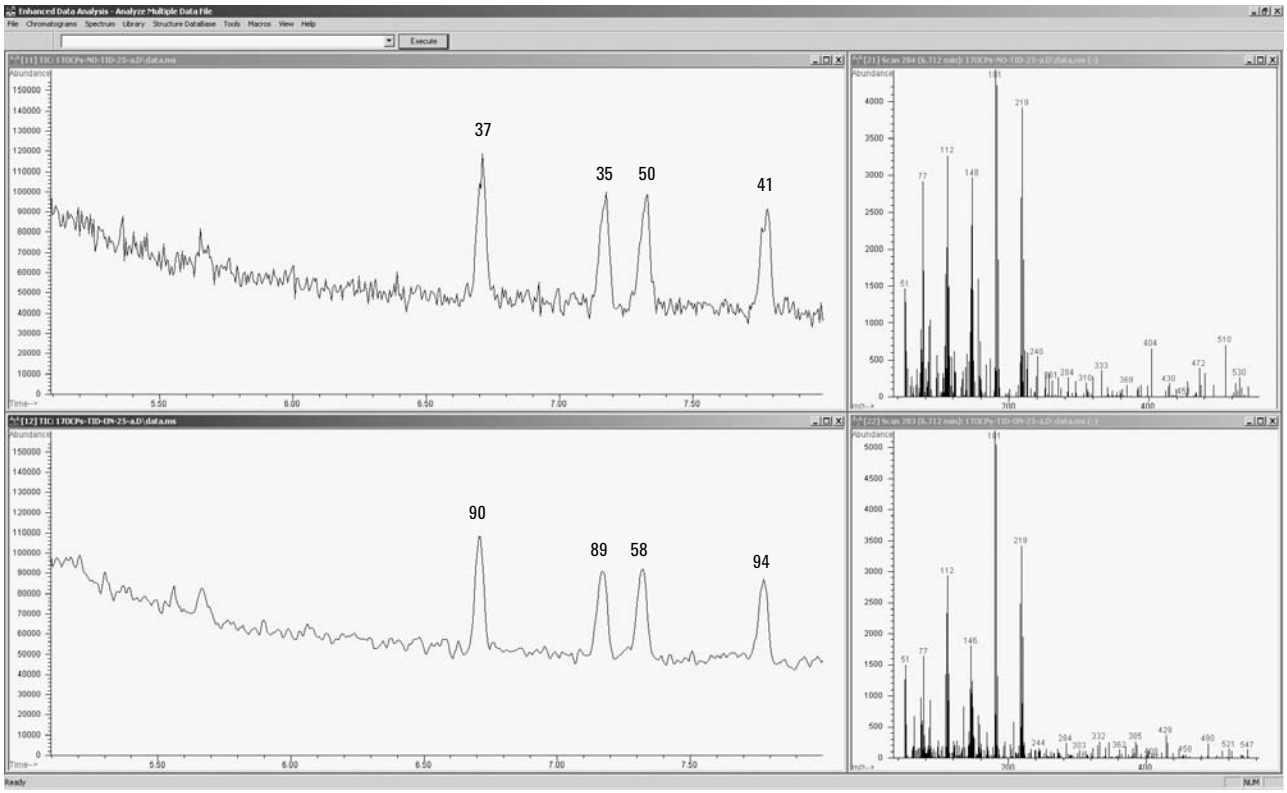

Figure 3. Reconstructed total ion chromatograms acquired with and without TID. Peaks are labeled with their PBM library search results: without TID (upper panel) and with TID (lower panel). Peak spectrum minus a fixed background range (5.5 to 6.5 minutes).

TID and Quantitation

The decrease of peak height with TID may also raise concerns about quantitation and detection limits, especially as signal height decreases with lower concentrations. Table 1 shows the relative increase in (rms) signal-to-noise (S:N) ratio for four model compounds acquired with and without TID (taken as the average of four acquisitions in scan mode). The increase in S:N ratio shows that TID better preserves signal while lowering noise at all concentrations. The enhanced S:N ratio at low concentration suggests better compound detection limits.

Table 1. For Various Concentrations, the Average Relative Increase in RMS S:N for Four Model Compounds Acquired With and Without TID (Average of four replicate scan acquisitions for each of the four compounds)

Concentration (pg injected)	25	50	100	250	500
Average relative increase in rms-S:N (n = 4)	20%	34%	79%	48%	61%

Another related aspect for compound detection limits, specifically quantitation limits, is ion area reproducibility or repeatability. Figure 4 shows the relative standard deviation (RSD) in ion areas versus concentration—with and without TID. Analysts are very familiar with the increasing variability in ion areas as compound concentrations decrease. This is due to several instrumental aspects, such as injection reproducibility, chromatographic concerns, etc., as well as compound spectral features which, in combination, dictate the compound quantitation limits. Figure 4 shows that for higher concentrations, the reproducibilities are high and RSDs are low and not significantly different between TID On or Off. But as the concentrations drops, reproducibility gets worse and RSDs increase. The filled markers indicate that TID maintains better ion area reproducibilities as the concentration drops, which results in better compound detection limits. Also, the improved ion profiles result in more consistent peak shapes and hence better peak integration results.

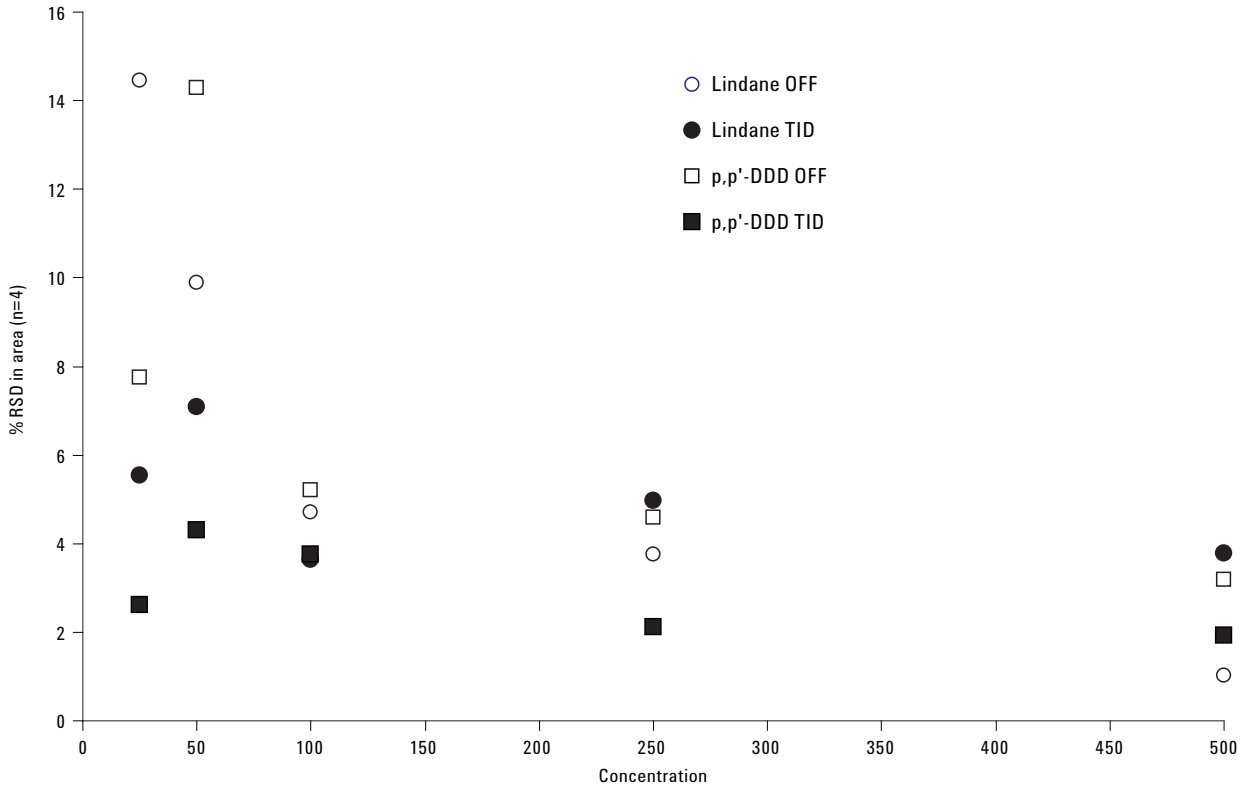

Figure 4. Relative standard deviation in quantitation ion area for two pesticides versus concentration: TID Off (open markers) and TID ON (filled markers).

Summary

Trace Ion Detection represents an innovative approach to enhancing compound detection and determination in GC/MS. By applying TID, the user can obtain improved ion profiles, which provide:

- Better peak shapes and therefore better and more consistent integrations
- Better spectral matches in complicated samples
- Improved signal-to-noise ratio for better detection limits
- Better ion area (and peak height) reproducibilities for enhanced detection limits

TID is most successful in improving quantitative analysis since those approaches tend to employ many samples over the chromatographic peak to better characterize the compound peak area or peak height used for quantitation. Usually this "over-sampling" of the peak creates a "noisier" signal and lowers spectral fidelity, etc. But TID better maintains analytical quality in these situations and creates a superior analytical situation.

Frequently Asked Questions About Applying TID

TID and Selected-Ion Monitoring (SIM): Can I apply TID in SIM?

TID sets a mode of acquisition and allows collection of SIM data. The same effects and results as cited above for scan will apply to SIM results. TID generates the best results when peaks have many samples over them as is the typical situation in SIM, which is designed for quantitation. Users applying compound peak heights (such as in pesticide analysis) will find improved peak height assignments even though they will appear lowered by TID.

Regardless, all data used for quantitation must be either TID On or TID Off and not some mixture to obtain consistent results.

What changes should I make in my GC/MS methods?

You can expect to lower your thresholds (in MS parameters) with TID enabled. Another benefit

worth exploring is narrowing the extracted ion window from the default of –0.3 to +0.7 to ± 0.3 or ± 0.4 *m/z*. TID creates more consistent mass assignments for ions, and the narrower window will make peaks less obscured by some interferences.

How should I explore adding TID to my methods?

You should look globally at your methods as advised in the Introduction. Performing gain-normalized tuning and optimizing the ion source temperature can dramatically improve methods. Examining low concentration standards and samples in replicate injections with and without TID will provide a good comparison. Also, examine narrower mass assignment windows with TID. Linear working ranges will be unchanged.

What should I look out for in applying TID?

TID peak shapes are slightly broadened and closely eluting compounds that share a common ion (for example, polyaromatic hydrocarbons) may show lower chromatographic resolution. While the chromatographic peak shapes may be broadened, they will also be improved in their shape, which should result in easier and more consistent integrations. In this way TID should produce superior data.

What methods will benefit most from TID?

Methods aimed at quantitation with many samples over the chromatographic peaks will show the greatest improvement. GC methods that employ constant pressure modes will benefit the most. Constant pressure allows the greatest reproducibility of compound retention times between GC instruments but is not an optimum mode for mass spectrometry. In constant pressure mode early eluting peaks are sharper (or narrower) than later eluting peaks because of the lowered linear velocity of the carrier gas as the oven temperature increases. Therefore, MS methods that have an optimum number of scans over the early eluting peaks will have too many scans over the later eluting peaks (unless the MS method is split up). The cost of too many scans over a compound chromatographic peak is lowered ion statistics. TID greatly ameliorates this issue.

References

Refer to the Agilent Web site: http://www.chem.agilent.com/Literature Library and select Online Literature.

1. Charles Thomson, Carolyn Broadbent, and Harry Prest, “The 5975C Series MSD: Guidance in Implementing High Ion Source Temperatures,” Agilent technical overview 5989-6051EN.
2. Jeffrey T. Kernan and Harry Prest, “The 5975C Series MSDs: Normalized Instrument Tuning,” Agilent technical overview 5989-6050EN.

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