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Sensitivity and noise in GC–MS: Achieving low limits of detection for difficult analytes

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Abstract

Gas chromatography–mass spectrometry (GC–MS) instrument limit of detection (LOD) is typically listed by major vendors as that of octafluoronaphthalene (OFN). Most current GC–MS instruments can achieve LODs in the low femtogram range. However, GC–MS LODs for realistic analytes in actual samples are often a few orders of magnitude higher than OFN's. Users seldom encounter 1 pg LOD in the single ion monitoring mode in their applications. We define this detectability difference as the "OFN gap." In this paper, we demonstrate and discuss how the OFN gap can be significantly reduced by the use of GC–MS with supersonic molecular beams (SMB). Experimental results were obtained with a recently developed GC–MS with SMB named 1200-SMB, that is based on the conversion of the Varian 1200 system into a GC–MS–MS with SMB. With this 1200-SMB system, the LOD of all types of analytes, including OFN, in real samples is significantly improved through the combination of: (a) enhanced molecular ion; (b) elimination of vacuum background noise; (c) elimination of mass independent noise; (d) elimination of ion source peak tailing and degradation; (e) significantly increased range of thermally labile and low volatility compounds that are amenable for analysis through lower sample elution temperatures; (f) reduced column bleed and ghost peaks through sample elution at lower temperatures; (g) improved compatibility with large volume injections; and (h) reduced matrix interferences through the combination of enhanced molecular ion and MS–MS. As a result, the 1200-SMB LODs of common and/or difficult compounds are much closer to its OFN LOD, even in complex matrices. We crossed the <1 fg OFN LOD milestone to achieve the lowest LOD to date using GC–MS, but more importantly, we attained LOD of 2 fg for diazinon, a common pesticide analyte. In another example, we achieved an LOD of 10 fg for underivatized testosterone, which is not amenable in traditional GC–MS analysis, and conducted many analyses of naturally incurred testosterone in alligator blood extracts. In comparison with standard GC–MS, we measured detectability enhancement factors of 24 for dimethoate, 30 for methylstearate, 50 for cholesterol, 50 for permethrin, >400 for methomyl, and >2000 for $C_{32}H_{66}$. In general, the harder the compound analysis, the greater is the gain in sample detectability using the 1200-SMB versus traditional GC–MS. Thus, the 1200-SMB lowers LOD, particularly for difficult analytes that are normally sacrificed in methods, and the detectability gains can amount to a few orders of magnitude over traditional GC–MS in real-world applications.

Keywords: Detectability; Sensitivity; Limit of detection; Gas chromatography–mass spectrometry; Supersonic molecular beam mass spectrometry

1. Introduction: detectability in GC–MS analysis

As most analytical chemists know, the term "sensitivity" in analytical chemistry is commonly misused, which has led to confusion and debate [1–4]. Sensitivity is defined as the change in signal versus the change in analyte concentration, or essentially is the slope of the calibration curve in an analysis. For lack of a better term, analytical chemists often mistakenly refer to sensitivity as "the ability to achieve low limits of detection (LODs)." In this vernacular, more or better sensitivity correlates to lower LODs, and less or worse sensitivity relates to higher LODs. However, sensitivity is only one of the components in the LOD equation and since it correlates with the ionization yield at the ion detector it can be arbitrarily increased through increased ion detector voltage (gain). In practice, such as in tandem mass spectrometry, true gains in LOD are more commonly achieved

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by reducing matrix interference (increasing selectivity) and other types of chemical noise such as vacuum background, than by increasing sensitivity.

To account for this distinction, we shall use the term "detectability" in this paper to refer to the analytical figure of merit meaning "ability to achieve low LODs." Thus, one instrument with higher detectability than another means that it has a greater ability to achieve lower analyte LODs. We are not the first to use this term, and in fact, a book has been written using the term in the exact same way [5]. We shall not use the term "sensitivity" unless we truly mean signal response versus amount of analyte.

Gas chromatography–mass spectrometry (GC–MS) is a central analytical technique that serves a broad range of applications aimed at sample identification and/or quantitative determination. Sample identification (qualitative analysis) entails a high degree of selectivity, while quantitative analysis requires high accuracy (precision and trueness). However, selectivity is very difficult to demonstrate in an instrument because there is no widely accepted scientific equation for selectivity in GC–MS. Thus, in practice, detectability is considered to be the most important GC–MS parameter and selling point because it stands out to characterize the quality and value of an instrument. There is good reason for this, as we describe below.

Superior detectability can be utilized in more ways than just for trace level analysis. Even if a given analysis does not require extremely low LODs, the extra detectability provided by an analytical technique can be translated into faster and easier sample preparation, more accurate data with improved reproducibility, superior sample identification, faster analysis and longer column and ion source lifetimes. For example, with significantly improved detectability, extraction can sometimes be avoided by using a direct sample "dilute and shoot" strategy. Improved detectability could enable the replacement of time-shared selected ion monitoring (SIM) with full scan mode, which enables library searching and increased confidence in sample identifications. Alternatively, splitless injections can be replaced with faster analyses using higher initial GC oven temperature and split injections, which may also lead to longer column lifetime. As a result, detectability is a key GC–MS specification highlighted by all the vendors.

However, *detectability is often misrepresented* since all the major vendors use octafluoronaphthalene (OFN) for their specifications in SIM and/or full scan reconstructed SIM (RSIM) modes. While the OFN SIM or RSIM specification is one way to compare the optimal instrumental detectability in standard GC–MS systems, the OFN specification is misleading when it is used to evaluate GC–MS LOD performance for real samples or when comparing different types of GC–MS instruments. In fact, detectability is not so straightforward, and actually it depends on a variety of factors, such as the analyte, matrix, analytical method, and cleanliness of the GC–MS system. OFN is uniquely easy for analysis, thus, it is ideal for the demonstration of the best case scenario for an instrument to achieve exceptionally low LODs. We maintain, however, that the detectability of an instrument should be judged by its performance using a mixture of several selected "hard to analyze" compounds, along with OFN.

In this paper, we discuss the term detectability and the full set of parameters that affect it, with examples from our GC–MS with supersonic molecular beam (SMB) system [6] (GC–SMB-MS). In this way, we intend to explore the various boundaries of GC–MS detectability, show what is required for its characterization in GC–MS, and highlight the technology of GC–SMB-MS and its improved performance with both OFN and real-world analytes and applications.

2. GC–MS with supersonic molecular beams: the 1200-SMB system

GC–SMB-MS is based on the use of an SMB for interfacing the GC to the MS [6–16] and as a medium for electron ionization of sample compounds [6,8,12,16,17]. SMBs are characterized by intra-molecular vibrational supercooling, unidirectional molecular motion with controlled hyperthermal kinetic energy (1–20 eV), mass focusing similar to that in a jet separator, and capability to handle very broad range of column flow rates from standard 1 ml/min (or lower) up to 90 ml/min [12,16]. We recently combined the benefits of an SMB interface and its related fly-through electron ionization (EI) ion source with the advanced features of the Varian 1200L GC–MS and MS–MS (Varian Inc., Walnut Creek, CA), resulting in a new and powerful GC–MS platform with record setting performance [6]. This new system, named 1200-SMB, is described in detail in ref. [6], thus, it will be discussed here only briefly. In the 1200-SMB, the column output is mixed with helium make-up gas (∼90 ml/min total), and flows to the supersonic nozzle through a heated and temperature controlled transfer line. The helium flow can be mixed (via the opening of one valve) with perfluorotributylamine (PFTBA) for periodic system tuning and calibration, or with methanol vapor for inducing cluster chemical ionization [18,19].

The sample compounds seeded in the helium gas expand from a 90 μm diameter supersonic nozzle into a nozzle vacuum chamber that is differentially pumped by a Varian Navigator 301 turbomolecular pump (Varian Inc., Torino, Italy) with 250 l/s pumping speed. The helium pressure at this vacuum chamber is about 6×10^{-3} mbar. The supersonic expansion vibrationally cools the sample compounds and the expanded supersonic free jet is skimmed by a 0.8 mm skimmer and collimated in a second differentially pumped vacuum chamber, where an SMB is formed. The second vacuum chamber is pumped by a Varian 300/400 split turbomolecular pump that pumps both the second vacuum chamber (400 l/s) and main MS vacuum chamber (300 l/s). The SMB seeded with vibrationally cold sample compounds fly through a dual cage EI ion source [20] where these beam species are ionized by 70 eV electrons with 10–15 mA emission current. The ions are focused by an ion lens system, deflected 90° by an ion mirror and enter a radiofrequency (RF)-only hexapole ion transfer optics (Q_0 of the original 1200 system). The 90° ion mirror is separately heated and serves to keep the mass analyzers clean from sample induced contaminations. The ions are further transferred through an ion lens into the MS vacuum chamber and are analyzed by a quadrupole

MS–MS mass analyzer system. It consists of two quadrupole mass analyzers (Q1 and Q3) and a collision cell (Q2). As any quadrupole MS–MS system, it can operate in SIM or full scan mode, as well as in all common MS–MS scan modes. Since Q2 is a 180° curved RF-only quadrupole ion transfer system in the 1200L, a head-on ion detector is positioned directly in the path of ions exiting Q3. Its entrance is biased at 5 kV, serving as an efficient ion to electron converter. Due to the combination of the 90° ion mirror and the 180° bend of Q2, the mass-independent neutral noise (produced by the ion source) was lower than one count per 10 s in the 1200-SMB system.

3. GC–MS OFN signal, detectability, and the "OFN gap"

As mentioned previously, OFN is a unique compound in that it is ideally suited for demonstrating the lowest GC–MS detection limits. It is a highly inert, semi-volatile, non-polar chemical that produces sharp GC peaks, and possesses a 70 eV EI mass spectrum with a dominant molecular ion representing 32.4% of its generated ions (normalized molecular ion mass spectral yield as calculated from its NIST library mass spectrum). OFN also has very low relative abundance of isotopomers around the 272 *m/z* molecular ion. Furthermore, the vacuum background noise in the 50–300 *m/z* mass spectral range has the lowest value at 272 *m/z*. As a result, OFN is the compound of choice by the main GC–MS vendors for instrument detectability specifications. Typical OFN specifications for advanced commercially available GC–MS systems are S/N > 100 using root-mean square (RMS) noise for 1 pg OFN in full scan mode with RSIM on *m/z* = 272, and/or S/N > 100 (RMS) for 100 fg OFN in SIM mode at *m/z* = 272. These values are "time dependent" yet they are only a little different from what was reported in ref. [21].

These OFN specifications create an expectation that GC–MS can easily analyze compounds at sub-pg on-column amounts, particularly in SIM mode. However, the common reality is that GC–MS users are unable to obtain sub-pg LOD for most of their samples in SIM mode. Many GC–MS users are familiar with this "OFN gap," and despite 100 fg OFN LOD specifications, analysts struggle to achieve a 50 pg injected LOD in SIM mode to obtain the minimum of three ions needed for pesticide analysis in complex agricultural matrices (1 μl injection of 5 g/ml extracts to detect 10 ng/g in the sample) [22]. This type of 500-fold discrepancy between the OFN specifications and real-world analyses must be properly explained in order to understand and evaluate the real GC–MS performance. LOD of samples in complex matrices is typically limited by chemical noise, even after extensive clean-up, not instrumental noise, and the key to achieving high-quality real-world results is to use instruments that minimize the effects of matrix on the analysis while maximizing the signal on the molecular ion and other high mass fragments. However, as will be described below there is more to detectability than the above two major factors.

Four main reasons are responsible for the worse detectability of typical analytes in comparison to OFN:

3.1. Lower molecular ion yields

Since OFN has 32.4% of its ions appearing at 272 *m/z* (its molecular ion), lower normalized molecular ion yields of common analytes can amount to more than two orders of magnitude relative signal reduction. Alternately, high mass fragments typically suffer from significantly increased mass spectral background and matrix interferences compared to the molecular ion.

3.2. Peak tailing

Column- and ion source-related peak tailing reduce the relative mass spectral signal of common analytes in comparison with OFN, and this factor can exceed an order of magnitude particularly for the least volatile and generally problematic analytes. Increased ion source temperature can alleviate this problem but with a penalty of a reduced molecular ion plus increased intra-ion-source degradation of thermally labile analytes.

3.3. Analyte degradation or retention

Many samples partially or fully degrade in the GC injector, column and/or ion source, or they do not elute in view of their limited volatility, and thus are lost in the column.

3.4. Condition of the instrument

OFN specifications as given by the various vendors are obtained with factory cleaned vacuum chambers of new systems. Recognizing that the molecular ion of OFN at 272 *m/z* already falls in a region with the lowest mass spectral noise even in the case of used systems, the vacuum background at 272 *m/z* can be <100 ions/s in new systems. In contrast, real-world systems typically exhibit significant vacuum background over a wide *m/z* range due a long history of complex sample injections, which can easily increase the mass spectral noise to >10,000 ions/s.

4. Detection of OFN using the 1200-SMB

One of the goals of this paper is to illuminate the few reasons as above for the OFN gap and discuss and demonstrate how it can be significantly overcome, particularly with the 1200-SMB. In Fig. 1, <1 fg LOD for OFN is demonstrated with the 1200-SMB. The Varian OFN test sample was used with an OFN concentration of 1 ng/ml and a 4:1 injector split ratio to yield ~200 fg OFN on-column. Our measured S/N using peak-to-peak noise was 320, which gave a linearly extrapolated LOD of 0.6 fg. We note that the Varian five-point RMS calculation software reported S/N of 7448 using RMS noise, but we believe that this is an unrealistic calculation of 0.13 fg (130 ag, assuming 5 RMS noise as the LOD) emerging from the sparse nature of the noise.

This issue of how noise is measured brings up another important discussion point. The computer software provides a consistent and precise approach in the measurement, but our view is that the values given are inaccurate. We feel that whenever the noise is in the form of sparse, occasional *single* ion noise (such as often encountered in MS–MS), a "manual" estimate of

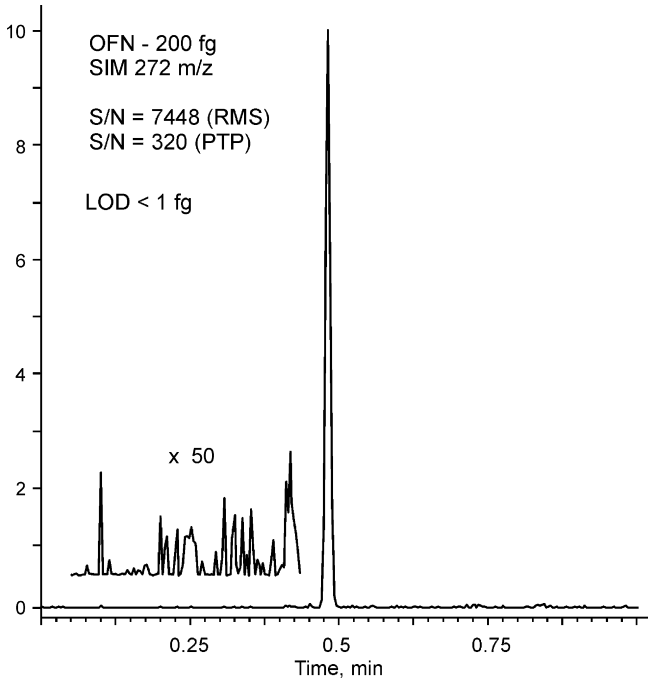

Fig. 1. A demonstration of <1 fg LOD of OFN with the 1200-SMB, in which 1 ng/ml OFN solution was injected (1 μl) with 4:1 split into a 4 m column with 0.25 mm i.d. and 0.1 μm VF5ms film; 4 ml/min helium column flow rate was used and the GC oven was programmed at 30 °C/min starting from 50 °C.

peak-to-peak noise should be preferred over computer generated 5 RMS noise for the estimate of LOD. We used a consistent approach in our noise measurements in which random peak-to-peak noise was taken, not including obvious spikes from chemical interferences in the chromatogram. The reader can repeat the measurements using the provided figures using their approach, but we tended to be conservative in our LOD measurements. Moreover, contrary to some suggestions, we believe that working with S/N in the order of ∼100 (in peak-to-peak) and to make a linear extrapolation to obtain the LOD estimate is superior to working with concentrations close to the LOD because possible carryover can lead to more biased results when working with very low sample amounts.

We used ion counting to calibrate the ionization yield and found that the 1200-SMB *sensitivity* was 10–12 counted OFN molecular ions (272 *m/z*) per femtogram injected. Coincidently, we also found that this is exactly the same sensitivity that we measured for OFN with the original 1200 GC–MS system before its conversion to the 1200-SMB. Thus, for OFN the use of SMB did not provide a gain in signal (sensitivity), but provided a modest gain in its detectability. In SMB-MS, there is only a negligible cooling-related gain in the relative yield of the OFN molecular ion since it is already the dominant ion for this relatively small and very rigid compound. Thus, the ionization yields at the entrance of Q1 are the same in the 1200-SMB and standard 1200 GC–MS systems.

On the other hand, the noise level of the 1200-SMB is significantly lower than typically obtained with the standard 1200 GC–MS, and thus we were able (as demonstrated in Fig. 1) to cross the record low 1 fg LOD milestone. Our noise was about

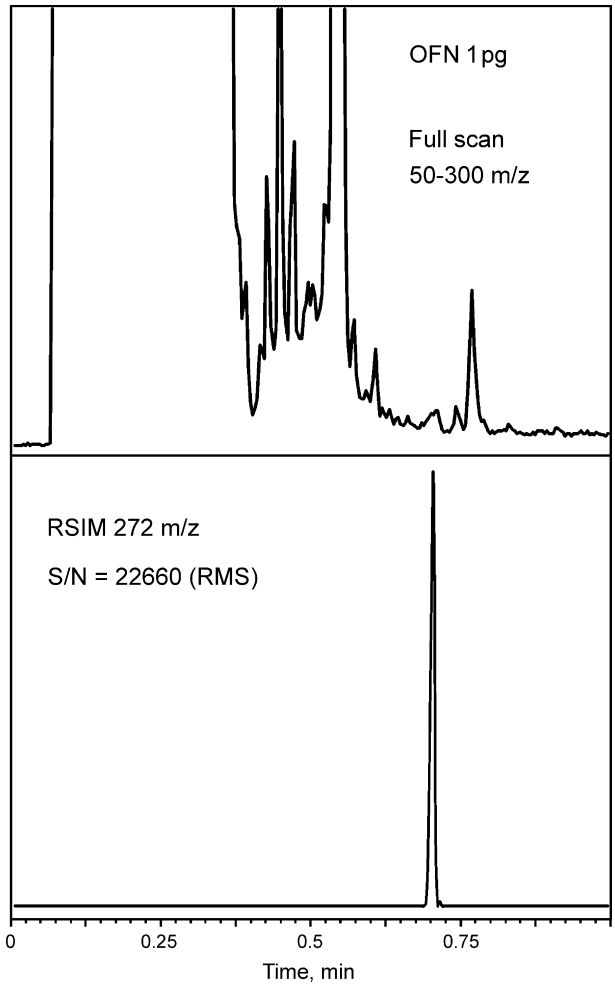

Fig. 2. Detectability demonstration in full scan RSIM of 1 pg OFN. Conditions are as in Fig. 1 except that 5 pg/μl OFN sample was injected and 2 ml/min helium column flow rate was used. The mass spectrometer was scanning at 5 Hz in the 50–300 *m/z* mass spectral range. The obtained S/N was calculated using the Varian software, but in fact it could be arbitrary increased with the ion detector voltage since it had no ion noise.

3 ions/s, which is less than 1 ion/dwell time. In comparison, the noise at $m/z = 272$ with the original 1200 GC–MS was typically 100 ions/s, and at best 20 ions/s. Thus, our OFN detectability record is attributed to record low noise as discussed below and not to improved signal (sensitivity).

We feel that the measured OFN signal (in ion counts per femtogram units) and noise (ion count/s) is the proper way of indicating GC–MS factors leading to low LOD (high detectability). Whereas the signal is usually stable for some time until the ion source requires cleaning, the noise strongly depends on vacuum background and system history, thus it is more variable. As a result, a quick evaluation of the noise situation of the instrument in combination with the previously recorded OFN signal enables the prediction of the system OFN detectability without having to actually make OFN injections.

In Fig. 2, the full scan total ion chromatogram of 1 pg OFN is shown (upper trace) together with its 272 *m/z* RSIM mass chromatogram (lower trace), analyzed at the conditions given in the caption. S/N was 22,600 as reported by the Varian software,

but this number is not meaningful since there is no noise from single ions. The actual S/N can be arbitrarily increased in this situation, consequently we can increase the signal by increasing the ion detector voltage to get a higher, but still false, indication of S/N > 1,000,000 (which was done experimentally). *It is important to note that the "pen limited" low noise in* Fig. 2 *is dominated by true electronic amplifier noise, and no zero offset was performed, thus any single ion could be detected* (*setting the zero offset above the noise level can falsely mimic this figure to make it look like there is no noise*). In full scan, the RSIM noise (which in this case corresponds to the number of occasional single ions/s) is reduced in comparison to SIM by the mass range (m/z = 50–300) divided by the mass resolution (0.7 m/z in this case), which led to ~360 times less noise in RSIM versus SIM. Thus, instead of having noise of 3 ions/s for OFN SIM at 272 m/z, we have a RSIM noise level at 272 m/z of one ion per 2 min. Consequently, we collected many RSIM chromatograms such as the one in Fig. 2 showing no (single) ion noise. Therefore, this type of instrument specification is hard to use, and it is meaningless for comparison purposes. We conclude and recommend that SIM should be used as a more meaningful way of expressing the detectability and S/N than RSIM.

Yue et al. recently reported a previously low record OFN LOD with their home-made GC–MS using time of flight (TOF) MS, which was operated with a unique EI ion source in superimposed magnetic and RF quadrupole fields [23]. They measured S/N of 8481 based on RMS noise for 934 fg OFN at 3.125 Hz (averaged spectra/s) and concluded from it a linearly extrapolated LOD of 0.33 fg. Our SIM (200 ms dwell time) LOD of 0.13 fg OFN using RMS noise was superior, but we believe that peak-to-peak noise is a better estimate for the LOD. However, the achievement of Yue et al. is impressive since it was obtained in the full scan mode. While our full scan RSIM S/N as demonstrated in Fig. 2 is superior to that of Yue et al. our high S/N value is misleading, since below about 30 fg, although the noise is zero, our full scan signal could also be zero.

However, the main concern with the very low OFN LOD of Yue et al. is that they also demonstrated peak tailing for OFN. While this tailing could emerge simply from using a poor GC column that induced peak tailing even for the inert OFN, it could also be attributed to the ion source structure. Their ion source has a very large internal surface to gas conductivity ratio, and as a result, each sample compound resides in it for many thermal adsorption/desorption cycles, which serves to increase the ionization efficiency, but also induces peak tailing (even for OFN at their reported 230 °C ion source temperature). We note that relatively closed ion sources are also used in internal ionization ion trap GC–MS, and while it can enhance the OFN detectability specification, such a closed ion source tends to: (a) deteriorate the chromatographic resolution for semi-volatile compounds (particularly polar ones); (b) require high ion source temperature, which promotes sample degradation; and (c) lower the relative abundance of the molecular ions. Consequently, we suggest again that GC–MS detectability should be defined with a mixture of sample compounds that may include OFN, but that should also include more realistic compounds that will more completely evaluate GC–MS detectability (akin to the famous Grob test mix for GC columns).

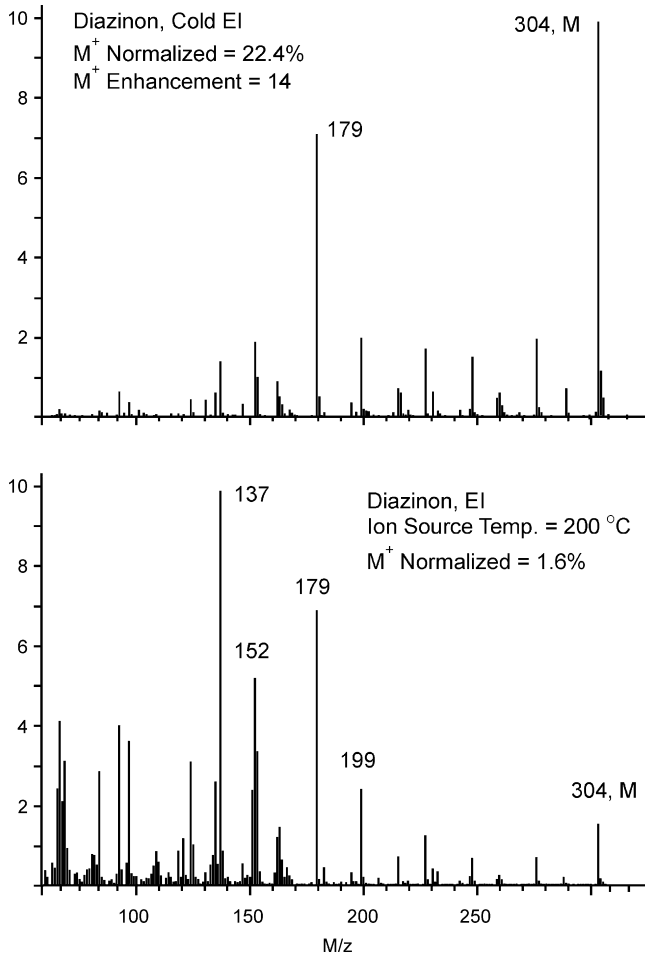

Fig. 3. Cold EI mass spectrum (upper) vs. standard EI mass spectrum (200 °C ion source temperature) of diazinon.

5. Closure of the OFN gap with the 1200-SMB

As mentioned above, OFN is a highly atypical molecule. For example, the pesticide, diazinon, which is an easy analyte in multiresidue methods, has only a 1.6% normalized molecular ion yield in standard 70 eV EI compared to 32.4% for OFN. *Note*: This 1.6% value for diazinon should not be confused with the 19% relative ion abundance of the molecular ion (304 m/z) in the mass spectrum (Fig. 3—bottom trace) because normalized yield is the ratio of a given MS ion peak to the sum of all peaks, as opposed to relative ion abundance, which is the ratio of the given peak to the base peak in the spectrum. Thus, standard GC–MS detectability measurements for diazinon using its molecular ion yield at least 20 times (32.4/1.6) higher LOD than for OFN. In Fig. 3, we show a comparison of the 70 eV EI mass spectra of diazinon obtained using standard GC–MS (the same Varian 1200L but with its original ion source) with 200 °C ion source temperature (bottom trace) and its cold EI mass spectrum obtained with the 1200-SMB (upper trace). The observed 14-fold gain in the normalized abundance of the molecular ion

Table 1
Ion source temperature effects on the TIC peak height *reduction factors* of the nine listed pesticides in comparison with the TIC peak heights obtained at 250 °C

Analyte	Ion source temperature (°C)		
	200	150	100
Dichlorvos	1.1	1.6	2.3
Dimethoate	1.1	2.3	10
Diazinon	1.5	2.3	6.5
Carbaryl	1.5	3.0	13
Folpet	1.3	2.5	7.0
Endosulfan	1.3	1.7	2.8
Piperonyl-butoxide	2.2	10	52
Permethrin	2.5	12	54
Deltamethrin	2.8	15	>50

The standard 1200 system was used as described in Fig. 4.

is attributed to the vibrational cooling of diazinon in the SMB. Since we found that the OFN signal is similar in standard 1200 GC–MS and 1200-SMB, this enhanced molecular ion directly induces at least a 14 times lower LOD with 1200-SMB for diazinon compared with standard 1200 GC–MS.

It should be mentioned that the signal intensity of the molecular ion also depends on the ionization cross-section of the selected compound. However, the ionization cross-section approximately depends on the number of electrons in the ionized compound and thus, for organic compounds it is approximately linearly increased with the compound molecular weight. On the other hand, such linear increase with the molecular weight is offset by the reduction of quadrupole mass analyzers transmission with mass (under constant resolution conditions) and the ion detector response could also be slightly reduced with mass. Consequently, these factors will not be further discussed.

However, the enhanced molecular ion is not the only aspect that increases detectability in the 1200-SMB. By using an ion source temperature of 200 °C in conventional GC–MS, another factor of 1.5 is sacrificed in diazinon analysis (compared with diazinon at 250 °C or OFN) due to tailing losses in the ion source. This leads to 30 times lower expected diazinon detectability than for OFN in traditional GC–MS. The effect of peak tailing on peak heights (due to metal surfaces in the ion source of the Varian 1200 GC–MS or other conventional GC–MS) is shown in Fig. 4 for nine pesticides at the ion source temperatures of 100, 150, 200, and 250 °C. In Table 1, the magnitude of this intra-ion-source peak tailing losses versus the 250 °C valued are listed for each pesticide. Note that even at 250 °C, some residual peak tailing losses occur for the late eluting pesticides, hence their peak heights are not maximized. Usually, the adverse effect of ion source peak tailing is underestimated because its appearance is deceiving in view of the fact that a sharp GC peak is retained, while most of the tail is like an iceberg in the form of a long, low amplitude tail that consists of a much greater percentage of analyte than occurs in the detected peak. In the inset in Fig. 4, the tailing peak shapes are clearly demonstrated for carbary. Similar results were found for all the nine pesticides with a gradual shift of the effect to higher temperatures for the later eluting pesticides. In addition to lowering detectability, peak tailing is also detrimental to the reproducibility of the analysis.

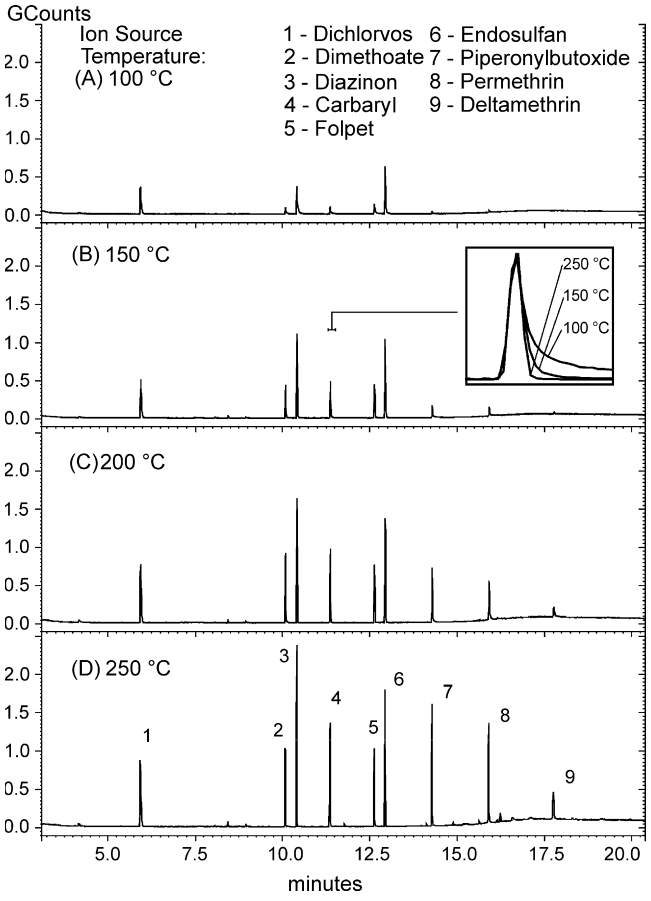

Fig. 4. The effect of intra-ion-source peak tailing on the GC–MS signal. The indicated nine pesticides were injected splitless at 10 ng/μl concentration using the Varian 1200L GC–MS with ion source temperatures as indicated. In the inset, normalized Carbary TIC peaks at the three indicated ion source temperatures are depicted to demonstrate the corresponding peak shapes.

While the ion source temperature can be increased to suppress peak tailing losses, this comes with a penalty of exponential loss in the relative abundance of the molecular ion for all analytes in the chromatogram [16,24,25]. Additionally, elevated ion source temperatures can adversely affect the library matching of the mass spectra [13] and detectability of thermally labile samples that tend to degrade on the metallic ion source surfaces. In Table 2, we compare the relative abundance of the molecular ion for the same nine pesticides shown in Fig. 4 and list in Table 1 using standard 1200 GC–MS at the different ion source temperatures and the 1200-SMB. One can see a gradual reduction in the relative abundance of the molecular ion with ion source temperature.

On the basis of the results shown in Tables 1 and 2, we conclude that by going from 150 °C standard 1200 GC–MS ion source temperature to 250 °C there is a *5-fold gain* in average GC peak height (thus in detectability) due to reduced ion source tailing, but this is offset and opposed by a *2.5-fold loss* in the average relative abundance of the molecular ion. This effect is even stronger for the more problematic analytes.

We note that any standard GC–MS EI ion source design provides several cycles of chemical adsorption/desorption from its walls, which are either inevitable or by design (in order to

Table 2
Ion source temperature effects on the *relative abundance* of the molecular ion (compared to the base peak) of the nine listed pesticides in standard 1200 and 1200-SMB

Analyte	Ion source temperature (°C)				SMB
	250	200	150	100	
Dichlorvos	3.6	4.0	6.0	7.0	22
Dimethoate	4.0	4.4	7.0	12	100
Diazinon	13	22	26	40	100
Carbaryl	2.1	3.4	5.0	6.8	20
Folpet	4.3	5.4	8.1	14	65
Endosulfan	0.1	0.6	1.4	2.9	15
Piperonyl-butoxide	0.4	0.7	1.7	2.8	10
Permethrin	0.1	0.4	0.8	1.9	10
Deltamethrin	0.1	0.3	0.4	–	5.5

increase the number of sample passes through the active ionization volume), which leads to unavoidable memory and peak tailing problems. In contrast, GC–SMB-MS with its dual cage fly-through ion source design [20] essentially avoids ion source memory and tailing, regardless of the sample volatility. Also, the ion source temperature becomes irrelevant to the relative abundance of the molecular ion. Furthermore, the fly-through ion source is inherently inert with no ion source degradation, yet the molecular ion is significantly more abundant than in standard ion sources, even at 100 °C since the vibrational temperature of the analytes is approximately −200 °C (∼70 K).

It should be mentioned that the feature of enhanced molecular ion with the 1200-SMB has a disadvantage in that the obtained mass spectra differ from standard EI mass spectra. This difference could impair database matching for identification purposes. However, we found in a comparative study [13] that while the matching to the NIST library were lower than with standard GC–MS, the probabilities of identification as reported by the NIST library were surprisingly higher than obtained with standard GC–MS for a group of 13 pesticides [13]. A possible explanation for this observation is that enhanced molecular ion also helps to better reject false identification. In addition, the obtained mass spectra with standard GC–MS such as the Agilent 5972 system (and especially in Leco TOF), suffer from a little lower molecular ion than in the NIST library. The reason for this is that the library mass spectra were typically obtained at lower ion source temperatures than used in real-world applications, in which the ion source temperature is increased for late-eluting analytes and for long term maintenance of the ion source cleanliness.

Based on these factors, we hypothesized for our experiments that diazinon would have a 20-fold lower LOD using the 1200-SMB (14-fold due to enhanced molecular ion and 1.5-fold due to peak tailing elimination) in comparison with standard 1200 GC–MS (GC peak width and chemical noise are not yet considered in our discussion). Fig. 5 shows the detection of 200 fg injected diazinon using the same column and conditions as for OFN in Fig. 1. An approximate S/N of 100 (peak-to-peak noise) was obtained (software calculated S/N = 1336 (RMS)), leading to linearly extrapolated LOD of 2 fg (0.8 fg using 5 RMS noise). The relatively small difference between LODs of OFN (0.6 fg)

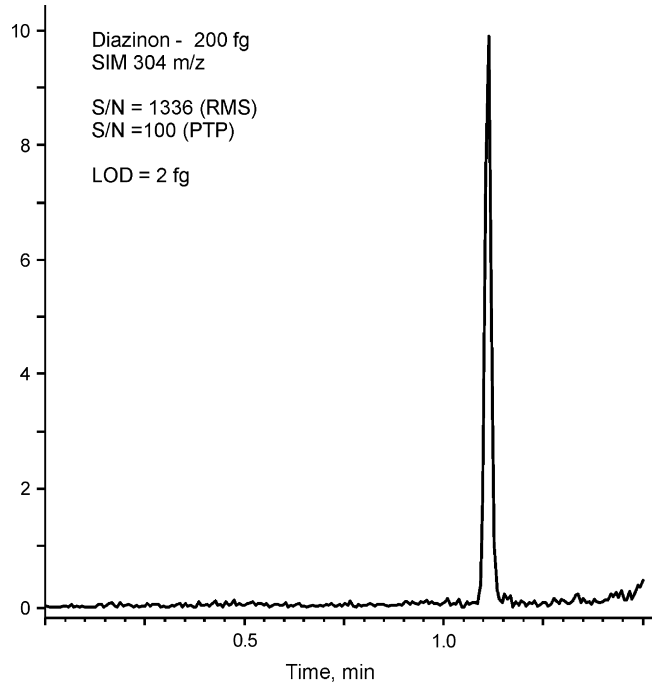

Fig. 5. A demonstration of 2 fg LOD of diazinon with the 1200-SMB. Conditions are as in Fig. 1 except that the sample was diazinon at 1 ng/ml concentration and the GC oven initial temperature was 120 °C.

and diazinon (2 fg) conforms rather well with the difference between their molecular ion yields in SMB-MS. In comparison, the standard 1200 GC–MS can only achieve a 2 fg LOD for OFN when the instrument is properly cleaned and fine tuned. Although we did not test diazinon LOD with the standard 1200 GC–MS, according to our measurements, we predict that it will be >30 times higher than for OFN due to the 20 times weaker molecular ion, factor of 1.5 peak tailing losses, and undetermined higher mass spectral vacuum background noise. Therefore, we estimate that the 1200-SMB is over 30 times more *sensitive* for a compound such as diazinon than standard GC–MS.

By the way, one may look at diazinon's mass spectrum in Fig. 3 and believe that the use the 179 *m/z* fragment for detection can lead to a 5-fold gain in signal over the molecular ion at 304 *m/z*. However, it was previously shown that every 100 *m/z* lower mass/charge for detection ions leads to 20 times increased matrix interference in complex samples [13]. Furthermore, MS–MS on the molecular ion was found to lead to a >20-fold lower LOD than with the 179 *m/z* fragment [6]. Thus, the molecular ion of diazinon is the best ion for its detection despite its lower abundance. Consequently, Figs. 1 and 5 demonstrate that the OFN gap is reduced by a factor of 20 with the 1200-SMB versus traditional GC–MS, and instrumental detectability is only 3 times lower for the real-world, non-halogenated analyte, diazinon, than it is for OFN.

In Figs. 1, 2, and 5, we measured the extrapolated LOD through the injections of 200 fg samples. This injected amount although small could seem to be too large for a linear extrapolation of the LOD. The injected amount was chosen to be 200 fg not only since this is the amount used in standard performance

testing of new GC–MS systems by a few vendors but mostly in order to escape the adverse effect of carry-over. We found that after a few analyses with nanograms injected amounts, standard GC–MS and our 1200-SMB system suffer from an injector carry-over problem that significantly contribute to the obtained peak heights in the low femtogram range. This small carry-over makes no difference in the peak height when higher amounts are injected. This effect is more noticeable for compounds with lower volatility than OFN and it is more important than column losses that could reduce the obtained peak heights. In addition, we wanted to use the same injected amount to better compare S/N using a series of compounds with different LODs, including testosterone (which has an LOD closer to the injected amount) as will be shown below for testosterone.

6. Achieving low limits of detection for difficult analytes

Approximately 30% of the chemicals in the NIST mass spectral library do not practically exhibit a molecular ion (<1% relative abundance) in their 70 eV mass spectra. Aliphatic compounds, such as $C_{32}H_{66}$, are characterized by a low ~0.1% relative abundance of the molecular ion in its mass spectrum and 0.03% normalized molecular ion mass spectral yield. For these types of compounds, one expects to measure 1000 times lower detectability (higher LOD in SIM mode on the molecular ion) than with OFN. In reality, these numbers are expected to be even worse considering peak tailing, vacuum background, column bleed, and ghost peaks as encountered in real working conditions. Note that the molecular ion is the only truly characteristic ion in the MS of most aliphatic hydrocarbons since their mass spectra are dominated by indistinguishing $m/z = 57$, 71, 85, etc., fragments. In Fig. 6, results obtained with the 1200-SMB are compared with results obtained with the standard 1200 system in the RSIM mass chromatograms of 10 ng cholesterol and $C_{32}H_{66}$. Note that the elution times with the 1200-SMB are significantly reduced in comparison with the standard 1200 GC–MS due to the use of shorter column and higher column flow rate [14]. We measured >50-fold higher detectability with the 1200-SMB for cholesterol ($m/z = 386$) than with the standard 1200 GC–MS, and for $C_{32}H_{66}$, no molecular ion of 450 m/z was detected in standard GC–MS, thus for $C_{32}H_{66}$ we measured >2000 times higher signal to noise ratio with the SMB-MS since our S/N was about 2000 in peak-to-peak units. The gain in S/N using RMS noise for both cholesterol and $C_{32}H_{66}$ was much higher than in peak-to-peak noise in view of the sparsity of noise from single ions (as shown in the figure insets). Thus, the detectability gain values above are based on peak-to-peak noise estimates as can be discerned from the figure itself.

Any detectability consideration must not ignore thermally labile and low volatility analytes, which either do not elute from the column or elute partially decomposed. They also often co-elute with extensive column bleed at the maximum column temperature plateau, and typically give very broad and/or tailing GC peaks. GC–MS detectability for these compounds can be >10,000-fold worse than for OFN (a huge OFN gap indeed!) or they can be undetected altogether.

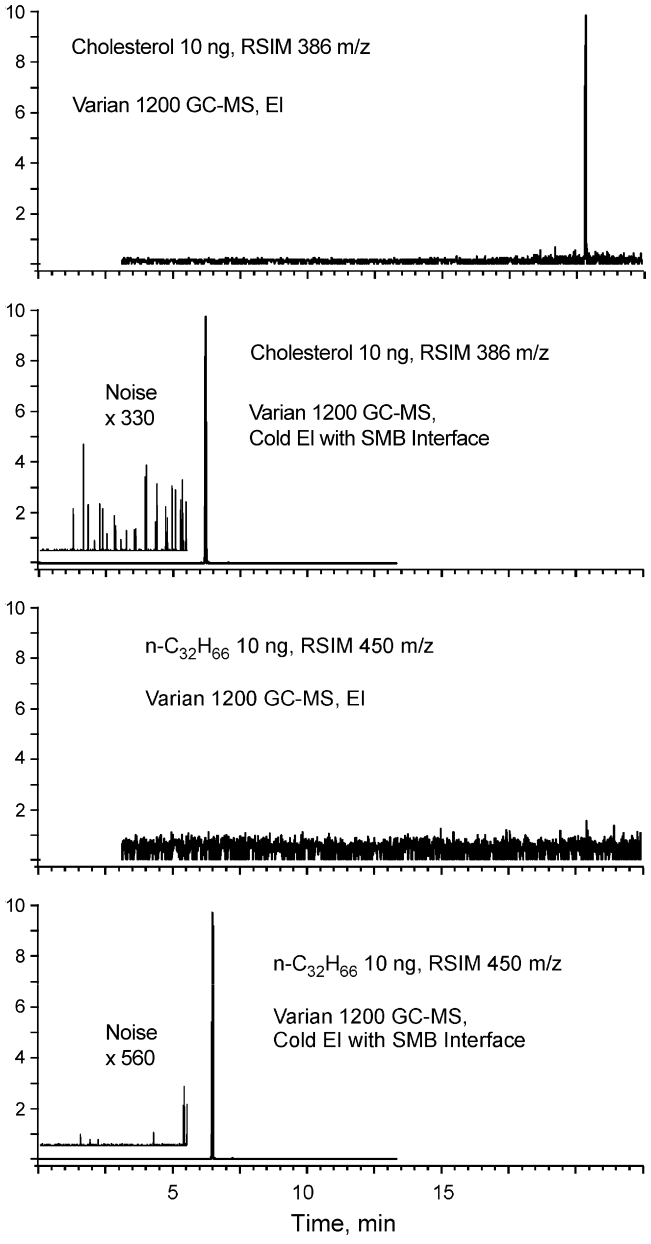

Fig. 6. A comparison of 1200 GC–MS and 1200-SMB in full scan RSIM of 10 ng each on-column of cholesterol and $C_{32}H_{66}$. The 1200 GC–MS was used with 30 m Varian VF5ms column with 0.25 mm i.d., 0.25 μm film thickness, and 1 ml/min helium column flow rate, while the 1200-SMB was used with the same column type but of 4 m length and 4 ml/min helium column flow rate. The vertical scale is in arbitrary units.

For example, testosterone is a steroid that is not amenable in traditional GC–MS analysis without derivatization. In Fig. 7, the 1200-SMB LOD for testosterone is demonstrated with similar column and conditions as for OFN (Fig. 1) and diazinon (Fig. 5). Testosterone has about 6% normalized molecular ion in its standard EI library mass spectrum and gives only a small molecular ion enhancement with the 1200-SMB (10% normalized yield). As shown, a 10 fg LOD of testosterone was achieved using peak-to-peak noise (or 2 fg for RMS noise using the Varian software S/N value of 667 for 200 fg). The detectability of the 1200-SMB for testosterone, an essentially undetectable ana-

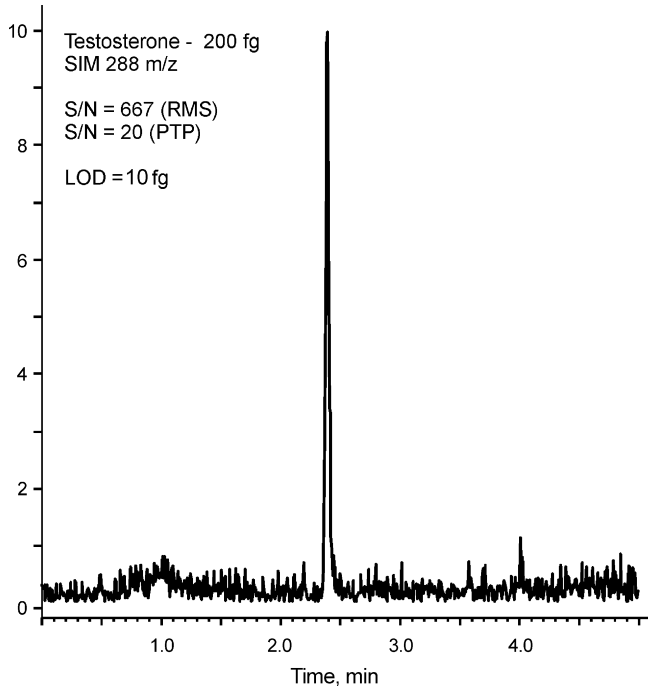

Fig. 7. A demonstration of 10 fg LOD of testosterone with the 1200-SMB. Conditions are as in Fig. 1 except that the sample was testosterone at 1 ng/ml concentration and the GC oven initial temperature was 120 °C.

lyte by traditional GC–MS, matched the OFN specification of traditional instruments. Similarly, the 1200-SMB can analyze a broad range of other thermally labile compounds, such as the carbamate pesticides aldicarb and methomyl [6,14].

$C_{72}H_{146}$ and similarly other low volatility compounds can be analyzed by the 1200-SMB at high column flow rates [6,14] whereas they cannot be analyzed by standard GC–MS. In these cases, an infinitely higher detectability is obtained for the very large aliphatic molecule $C_{72}H_{146}$ using its molecular ion of $m/z = 1011$ versus any other GC–MS system. This group of large, non-volatile analytes contains large PAHs, fullerenes (including C_{60}), triglycerides, polymers and other compounds. Even if such compounds could elute with standard GC–MS (retention times would be on the order of hours at 1–2 ml/min He flow rate), their analysis would be hampered by broad GC peaks, high column bleed, ghost peaks, ion source losses and tailing, partial decomposition, weak molecular ions, etc. All these problems are either eliminated or alleviated with the 1200-SMB. Consequently, GC–SMB-MS provides value in the analysis of analytes previously not able to be detected in GC–MS [14], and moreover can achieve low LODs for them, narrowing the OFN gap.

Thus, as mentioned above, GC–MS detectability cannot be properly judged by OFN specifications alone, and the practical detectability of the instrument is better represented by a broad range of analytes.

7. Noise—the hidden contributor to the OFN detectability gap

Low mass spectral noise is an important need in GC–MS applications, yet no vendor provides a specification for it, or discusses how it is affected by sample matrix, analytical methods, system history, ion source design, or pumping speed of the vacuum system. While the noise at $m/z = 272$ for OFN is very low, upon repeated use of GC–MS with real-world complex extracts, vacuum background accumulates and the noise level increases by as much as a few orders of magnitude.

When MS noise is concerned, not all GC–MS systems are the same. In the 1200-SMB, there is no mass spectral vacuum background and the noise can be below 10 ions/scan at all times, essentially independent of system cleanliness

Up to now, we have discussed the detection of analytes in simple, solvent-only matrices and the various factors involved, but the role of mass spectral noise and its various aspects cannot be ignored. In order to better understand the subject of mass spectral noise, one needs to divide the noise according into its several major components. A list of noise sources in GC–MS is included in the following paragraphs.

7.1. Vacuum background

This is the dominant mass spectral noise source at <400 m/z for all standard GC–MS systems. Furthermore, it is significantly increased after the injection of dirty matrices. The reason for this noise type is that while the ion source is heated to reduce the residence time of chemicals eluting from the GC column to <1 s, these compounds migrate from the hot (e.g., 200–250 °C) ion source to the much cooler (≈25 °C) vacuum chamber and pump mouth, where they reside for many hours, days, months, or even years (depending on the volatility of the chemicals). Eventually they are pumped away (higher pumping speed helps), but in the meantime they diffuse back to the ion source and create vacuum background mass spectral noise. In fact, the perfluorotributylamine (PFTBA) signal itself, which serves for MS tuning and optimization is a type of vacuum background as it migrates to the ion source by diffusion in a similar way as vacuum background noise.

We evaluated the vacuum background noise of an Agilent 5973 MSD system that is used for routine pesticide analysis in agricultural matrices at the Israel Plant Protection Center, and found that in the scan range of 50–700 m/z at 2 Hz, the mass spectra showed about 25,000 ions/scan and about 1500 ions at the most abundant ion of $m/z = 57$. The noise was distributed as 12,000 ions/scan in the 50–100 m/z range, 7200 ions/scan from 100–200 m/z, 2600 ions/scan from 200–300 m/z, 1250 ions/scan for 300–400 m/z, and 1000 ions/scan in the 400–500 m/z range. This amount of noise emerges from about 1 ng vacuum background compounds entering the ion source per second. If the MS vacuum chamber is contaminated with 1 mg sample matrix, and it crosses the ion source 10 times before its removal by the pump, such 1 mg can create the above-mentioned noise for over three months. Assuming equal mass distribution, the 2600 ions/scan in the 200–300 m/z range can be translated into $2600 \times 13 = 34{,}000$ ions/s averaged SIM noise for mass peaks in this range (at 2 Hz, the MS spent 1/13 of the time at the range of 200–300 m/z). Such noise is 340 times higher than typical factory-measured OFN noise, hence it could increase the LOD by an additional factor of 18 over OFN ($\sqrt{340}$).

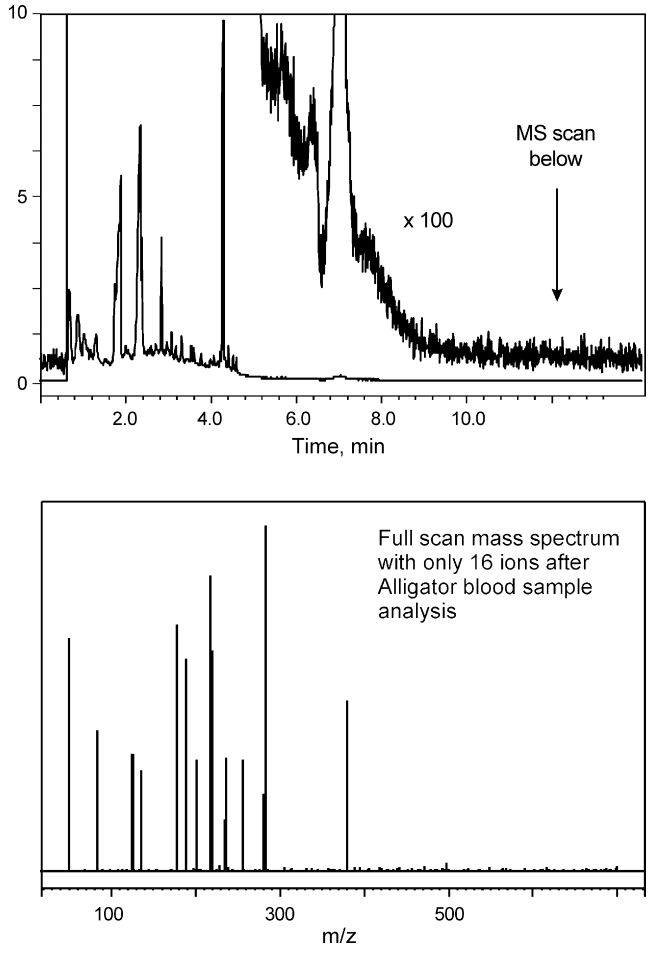

Fig. 8. A demonstration of very low MS full scan noise after the analysis of testosterone in alligator blood (plasma) extract after the GC column was cooled back to 120 °C. All the observed "peaks" in the mass spectrum arose from single ions being detected while the minor baseline noise is electronics noise (independent of the ion detector voltage). The mass spectral range was 50–700 *m/z* and scan rate was 2 Hz.

The 1200-SMB is the only GC–MS system in which mass spectral vacuum background is fully eliminated with its fly-through ion source [17,20]. In order to demonstrate this experimental finding, the 1200-SMB background was evaluated one day after 20 injections of alligator blood extracts (a very complex matrix), and we measured less than 20 ions per 50–700 *m/z* scan as mass spectral background. In Fig. 8, the full scan total ion chromatogram (TIC) of alligator blood extract is shown (upper trace) together with a magnified section of the TIC and a typical mass spectrum obtained at the end of the run (at the indicated time), after the column was cooled to its initial temperature of 120 °C. The most important information derived from Fig. 8 is that the mass spectral noise level is the same before and after the elution of this "dirty" matrix, and that its magnitude is exceptionally low (<20 ions/scan). In the lower trace each spectral line originates from a single ion, and the assigned masses almost randomly change every scan.

Most GC–MS users have never seen such low vacuum background mass spectra that are void of reproducible mass peaks unless the system is out of tune. The reason for this low noise in SMB-MS is that the dual cage ion source is uniquely characterized by zero electric field inside the ion cage and a small electric field barrier at its exit lens system [20]. Thus, vacuum background ions, with about 0.1 eV ion kinetic energy, do not exit the ion cage, whereas ions formed from molecules in the SMB (from the GC eluent) move forward with the same kinetic energy as neutrals in the SMB (a few eV) and fly through it into the mass analyzer [20]. Note that the mass spectral noise of ~20 ions/scan shown in Fig. 8 was obtained with a 260 °C transfer line and nozzle temperature. When the transfer line temperature was reduced to 100 °C, which is appropriate for OFN analysis, we measured only 8 ions per 50–700 *m/z* scan to obtain <10 ions/scan.

7.2. Mass independent noise

This type of noise originates from metastable helium atoms produced in the ion source, stray electrons, and vacuum ultraviolet (VUV) radiation. It accounts for ~100 ions/s in some standard GC–MS systems, and at 200 ions/s, it constituted the major noise source in our earlier supersonic GC–MS system based on the 5972 MSD [12]. However, in the standard 1200 GC–MS and 1200-SMB systems, this noise source is fully eliminated by the curved Q2 (and additional 90° ion mirror in the 1200-SMB). Guckenberger et al. [26] reported the reduction of this noise source to 2 ions/s by the curved quadrupole pre-filter in ThermoElectron's DSQ GC–MS. However, it did not help in their OFN LOD specification due to the dominant role of vacuum background noise in their system.

7.3. Transfer line and gas line impurities

This noise source is usually ignored since it is much lower than vacuum background noise, but it exists in all GC–MS systems because the transfer line is a part of the vacuum system in GC–MS. This type of noise is the major noise source in the 1200-SMB GC–MS since impurities arising from the transfer line become a part of the SMB (and are not suppressed by the fly-through ion source as in the case of vacuum background). Therefore, appropriate cleanliness of the transfer line and make-up gas line is important in the 1200-SMB and the helium make-up gas is cleaned with gas impurity traps just as the GC carrier gas (activated carbon black/charcoal). The major positive attribute of this noise type is that it does not relate to the injected samples, hence it is unaffected by the system's usage history (as demonstrated in Fig. 8) because the make-up gas in the SMB-MS transfer line does not contact the sample except at the inert Vespel heated nozzle, which has a small volume that is flushed by 90 ml/min helium.

Thus, even after the injection of very dirty matrices, the noise in the 1200-SMB remains as low as it was before the injections, and it can be >1000 times lower than in standard GC–MS in number of ions/s. This noise reduction factor results in up to 32 times lower LODs due to lower noise in routine analytical conditions.

7.4. Column bleed

Column bleed is a major source of noise for late-eluting analytes in GC–MS, and is the impetus for chromatography companies to try to develop and market columns with the lowest bleed. Column bleed is particularly problematic in the full scan mode, but in many cases, it also adversely affects SIM LODs. For example, column bleed is the reason why the vendors somewhat recently replaced hexachlorobenzene with OFN as their specification test compound. Column bleed increases in direct proportion with column length and film thickness, and exponentially increases versus column temperature. With the 1200-SMB, the GC elution temperatures are reduced through the use of high column carrier gas flow rate [14], thereby practically eliminating column bleed. Additionally, thinner films can be used to further reduce column bleed without a sacrifice of the column capacity when high column flow rates are employed. The reason for this is that the column capacity linearly increases with the column flow rate due to increased separation plate height when flow rates above the van Deemter optimum flow rate are used [14].

Fig. 9 shows the practical elimination of column bleed in the analysis of the indicated compounds. Increasing the column flow rate from 1 to 4 ml/min in combination with reduced column length from 30 to 4 m resulted in the lowering of elution temperatures for cholesterol and $C_{32}H_{66}$ from 320 to ≈220 °C, thereby practically eliminating column bleed and generating the observed flat baseline, independent of GC oven temperature. Note also that methomyl, a thermally labile carbamate pesticide, is detected with the 1200-SMB while it is fully decomposed in the standard 1200 GC–MS chromatogram.

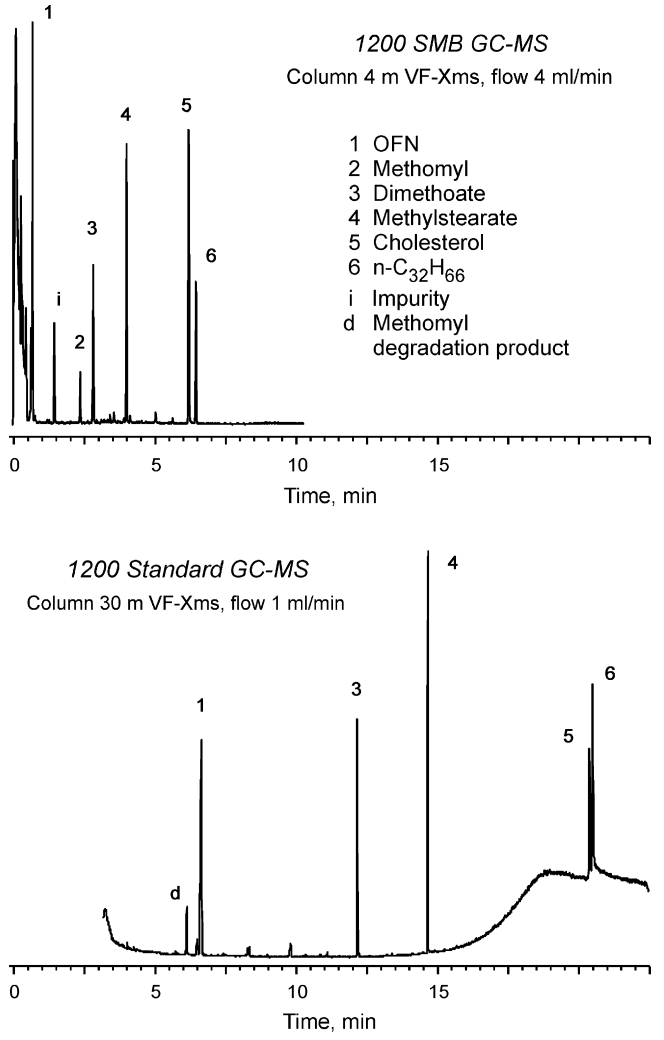

Fig. 9. The effect of increased column flow rate on lowering the elution temperatures of the indicated compounds and the elimination of column bleed. The 1200 GC–MS was used with a 30 m Varian VF5ms column with 0.25 mm i.d., 0.25 μm film thickness, and 1 ml/min helium column flow rate, while the 1200-SMB was used with the same column type but with a 4 m length and 4 ml/min helium column flow rate.

7.5. Ghost and septa peaks

Ghost peaks emerge from the elution of compounds that were injected in previous runs. Ghost peaks result in extended broadband mass spectral noise that is hard to subtract, and they are the main reason for GC methods to end with extended hold times at high GC oven temperature. The lower elution temperatures achieved with the 1200-SMB through the use of high column flow rates also mean that ghost peaks represent a smaller problem as they have higher chances of eluting in the first run. The reason for this is that with higher column flow rate, the matrix compounds also elute at lower GC oven temperatures, the same as sample compounds, and as a result, they have much greater chance to elute before the end of the first run without their conversion into ghost peaks at the next run. Injector-related septa bleed can also contribute to extra GC peaks and noise, but it can be reduced through the use of a temperature programmable injector, periodic liner maintenance, better septa, and an autosampler that introduces fewer septa pieces into the injector liner.

8. Matrix interference noise and its reduction

Matrix interference is a very important type of noise that is usually the limiting source of noise in real-world applications, thus deserving special consideration and its own section. In those many cases when matrix interference is the dominant noise source, the instrumental detectability becomes irrelevant to any improvements in LOD, and only the reduction of matrix interference can increase analyte detectability (lower the LOD). Furthermore, if matrix interference is not the limiting source of noise, one can increase the injected sample volume or use a more concentrated extract to further improve the detectability until reaching the matrix interference limit or until column and liner contamination become the method limitation. Six approaches to reduce matrix interference will be advanced in the subsections below.

Compiled from refs. [22,27–31], Table 3 demonstrates and compares the relative effects of the improved GC–MS instrument selectivity factors on analyte detectability in the real-world application of pesticide residue analysis in foods. The LOD values from the cited papers in Table 3 were converted to pg injected to yield reported S/N = 3. When the authors reported limit of quantitation (LOQ), which is traditionally defined as

Table 3
Estimated LODs (S/N = 3) reported in the literature converted into picograms injected using different GC–MS techniques in the analysis of pesticide residues in food

Pesticide	3-Ion SIM [22]	1-Ion SIM [27]	Full scan (RSIM) ion trap MS [28]	Ion trap MS–MS [28]	GC-TOF [29]	GC×GC-TOF [29]	HRMS w/TOF [30]	Triple quad MS–MS [31]
Acephate	380	180			6	0.9	8	
Captan	970	85			25	4	3	
Carbaryl	36	2			4	0.4	0.8	
Carbofuran	48		14	7				
Chlorpyrifos	36	2	28	0.2	3	1	0.2	
DDE	24		14	1				0.05
Deltamethrin	400	20			84	60	0.8	
Diazinon	24		4	0.4				
Dichlorvos	36	0.5			6	0.1	0.3	
Dimethoate	36	3			2	0.9	0.8	
Endosulfan	24	15			12	4	0.8	0.5
Endosulfan sulfate	97	3	120	7	8	5	0.3	0.5
Heptachlor	24	2			5	0.6	0.2	0.2
Lindane	24	3			9	0.5	0.2	0.05
Metalaxyl	24		45	7				
Methamidophos	120	10			4	1	0.8	
Methiocarb	73	2			4	0.9	0.8	
Parathion-methyl	36		10	0.3				
Permethrin	48	10			18	12	1	
Phosalone	61	2			8	4	0.2	
Pirimiphos-methyl	24	1			6	1	0.3	
Procymidone	36	2			9	0.4	0.8	
Propargite	24	2			11	4	3	
Quintozene	24		10	0.4				
Vinclozolin	24		14	0.2				

Ref. [22], acetonitrile extraction of fruits and vegetables, 2 g/ml, 2 μl injection, LOQ/3.3; ref. [27], acetone extraction of carrots, 5 g/ml, 1 μl injection; ref. [28], supercritical fluid extraction of fruits and vegetables, 1.2 g/ml, 3 μl injection; ref. [29,30], ethyl acetate extraction of peaches, 1 g/ml, 1 μl injection, LOQ/3.3 for [30]; ref. [31], 1/1 ethyl acetate/cyclohexane extraction of fat, 0.125 g/ml, 4 μl injection.

the concentration at which S/N = 10, rather than LOD, the values were divided by 3.3 in Table 3. Different instruments, GC injector and separation conditions, sample preparation approaches, matrices, injection volumes, concentration factors, and LOD estimation procedures were used by the different authors, which makes the comparison imprecise, but the general conclusion about the relative effectiveness of each approach is valid. In general, Table 3 demonstrates the fact that GC–MS systems with enhanced selectivity are characterized by lower LODs. Table 3 also demonstrate with additional numbers the OFN gap through showing LOD values for real pesticide that are several orders of magnitude higher than OFN specifications that are given by the vendors. In addition, the reported LODs span about two orders of magnitude range for each system and method while failure (too high LODs) are usually not reported.

8.1. Improved sample preparation

This action is often the first choice of GC–MS users when matrix interference is the limiting source of noise because the analysts have some control in this aspect. For example, the same sample preparation method was used in only two of the references among the six cited in Table 3 [22,27–31] for a similar application. The recent development of the QuEChERS method [32–34] helps in our experience to significantly clean agricultural extracts for analysis. However, the need for improved sample cleanup is often costly and laborious, and thus instrument-based alternatives are highly desirable.

8.2. Enhanced molecular ion

The effect of matrix interference can be reduced by enhancing the molecular ions of the analytes. We found that average matrix interference in complicated samples is reduced 20 times for each 100 *m/z* mass increase [13]. Every EI mass spectrum has relatively sparse ion peak density near the typical molecular ion of 200–500 *m/z*, while in the 50–150 *m/z* range, many mass spectra often show peaks (noise from the matrix) at every *m/z* value. While it is common knowledge that matrix interference is reduced at higher *m/z*, the large magnitude of this effect implies that the enhanced molecular ion exhibited in the 1200-SMB provides an effective way to reduce matrix interference chemical noise, as discussed at length in ref. [13].

8.3. Selective ionization methods

Selective ionization methods, such as positive ion or negative ion chemical ionization (PICI or NICI), hyperthermal surface ionization, photo-ionization, etc., by their nature effectively ionize the sample while minimizing ionization of some or most of the matrix (otherwise they would not be used). However, the main drawback of these methods, other than added cost, is their

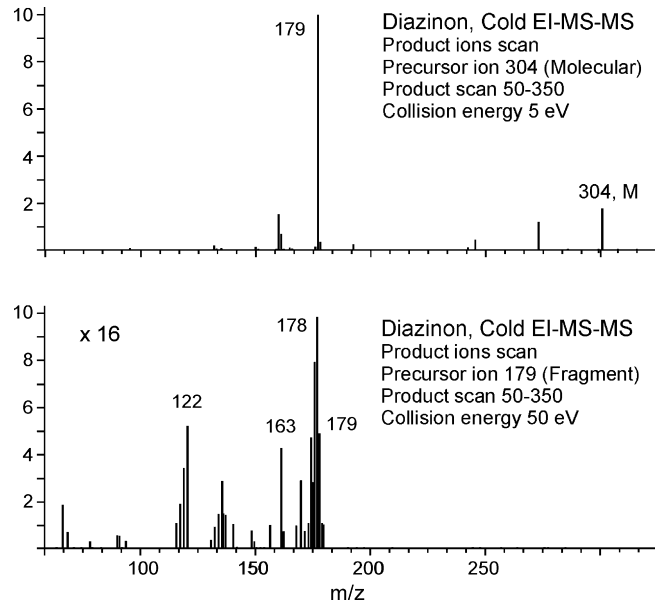

Fig. 10. A comparison of MS–MS spectra of diazinon obtained on its $m/z = 304$ molecular ion with 5 V CAD energy, and on its major fragment ion ($m/z = 179$) with 50 V CAD energy.

limited applicability. Also, some instruments require extensive modifications to use PICI or NICI.

8.4. MS–MS

MS–MS is often the method of choice used to suppress matrix interferences in targeted compound analysis [28,31]. MS–MS is focused on mass spectral noise reduction, both matrix and vacuum background, with a small penalty in signal reduction. However, MS–MS has two major downsides: (a) added instrumental cost and/or complexity and (b) MS–MS focuses on targeted analytes only, and cannot be effectively used for the analysis of unknowns. MS–MS is by now a well-established technology which is used in most LC–MS systems and in a portion of GC–MS systems. While its success in the reduction of matrix interference is overwhelmingly documented, much less is known about how much it reduces matrix interference and how universally applicable it is.

In our experience, we found that MS–MS is favorably applicable to about 85% of the samples that we tried. For some compounds MS–MS fails when it results in very weak product (daughter) ions. We found that MS–MS typically lowers matrix interference by about a factor of 2–20, in agreement with literature values for pesticide analysis [28]. We further found that the efficiency of MS–MS is much better using the molecular ion as the parent because it typically requires lower collisionally activated dissociation (CAD) voltage and has a higher yield of at least one major transition ion. In Fig. 10, >20-fold improved MS–MS product ion yield of diazinon on its molecular ion versus on its $m/z = 179$ MS fragment is demonstrated. Usually, the molecular ion is easy to fragment by CAD at low collision energies, while the high intensity fragment that is formed is more stable (this is the reason why it was formed), thus harder to dissociate. Consequently, higher collision energies are needed to

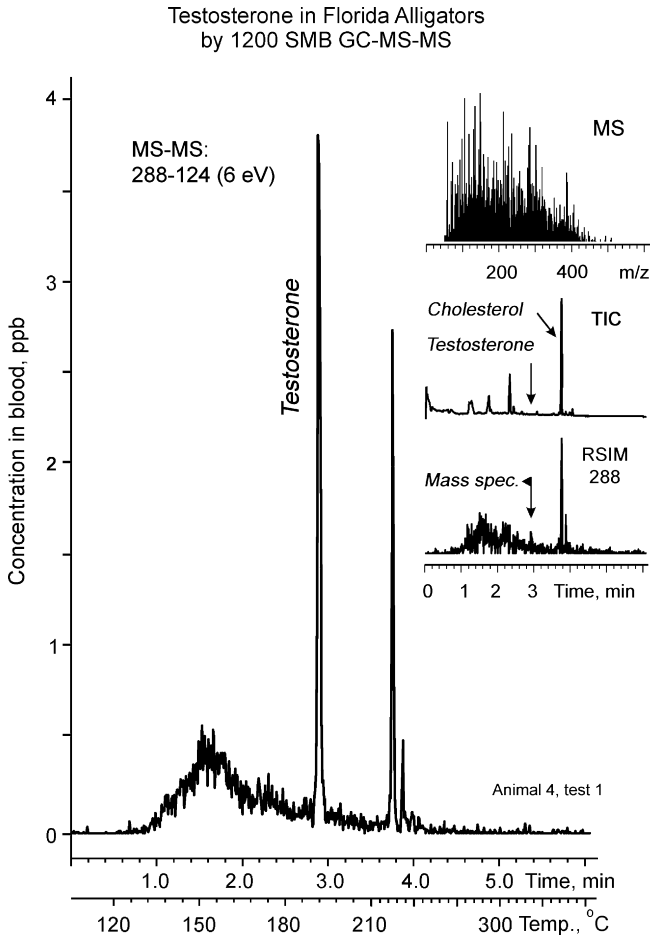

Fig. 11. The analysis of testosterone in Florida alligator blood with the 1200-SMB; 4 m Varian VF5ms column with 0.25 mm i.d., 0.1 μm film thickness and 12 ml/min helium column flow rate was used. MS–MS was employed on the molecular ion ($m/z = 288$) with 6 V CAD energy and $m/z = 124$ detected as the product ion. The right side insets were taken from another run and are mass spectra at the testosterone elution time, TIC, and RSIM at $m/z = 288$ for testosterone.

decompose the fragment, and when it is dissociated, it degrades into many smaller fragments (see Fig. 10) which are formed at higher kinetic energies and not efficiently detected. Thus, MS–MS is generally more selective and yields lower LOD using the molecular ion as the precursor (parent), and the 1200-SMB feature of enhanced molecular ion and peak tailing elimination improved the MS–MS detectability considerably.

In Fig. 11, the quantitative analysis of testosterone in blood plasma extracts from Florida alligators is shown using MS–MS of the testosterone molecular ion (288 m/z) with 124 m/z as the monitored product ion. Alligator blood extracts were given to us as a challenge using GC–SMB-MS for these measurements [35]. A good S/N ratio of ~120 was obtained for 3.8 ng/g incurred testosterone in the alligator blood, to yield a method LOD of <100 pg/g (ppt). The injection volume was 1 μl of of relatively unconcentrated 1 g/ml alligator blood extract since higher extract amounts (concentrations) saturated the column and started to shift the testosterone elution times. At the upper right side of the figure, the complexity of the mass spectrum at the testosterone elution time is shown, taken from another full

scan run, and the RSIM at 288 *m/z* is shown below it, demonstrating the significant reduction of matrix interference obtained by MS–MS. We found that for this sample, MS–MS (with 1.2 *m/z* resolution) resulted in 14 times lower testosterone signal (sensitivity) compared to SIM, but with about 20–25 times better testosterone detectivity due to matrix noise reduction. While this feature of MS–MS is well-known and documented [28,31], the 1200-SMB is the only GC–MS technique that can analyze such trace levels of thermally labile analytes in complex matrices as demonstrated in Fig. 11 for testosterone, which is incompatible with standard GC–MS analysis without derivatization.

8.5. High resolution GC–MS

High resolution (HR) MS is available today with each type of major mass analyzer. Clearly, increased MS resolution reduces matrix interference through improved MS separation capability [30]. For mass analyzer resolution of 10,000, the peak width at 300 *m/z* is about 0.03 amu. We analyzed the accurate masses of the full list of 628 NIST library compounds with 304 amu and found that their accurate mass distribution width was 0.166 amu around 304.123 amu (standard deviation of 0.083 amu). Thus, we found that for diazinon as an example ($C_{12}H_{21}N_2O_3PS$, 304.10 *m/z*) HRMS with resolution of 10,000 improves the selectivity by an average factor of 9 (4.5 for resolution of 5000). While such a modest factor of 9 applies to the majority of compounds with CHNO atoms, in rarer cases such as tetrachlorodioxins, which are characterized by a large negative mass defect (due to four chlorine atoms and relatively few hydrogen atoms), HRMS significantly improves the selectivity.

For example, Cajka and Hajslova [30] explored the advantages and limitations of GC-HRMS with a TOF instrument in a real-world pesticide residue application. For a multi-chlorinated analyte, such as chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$), they reported a 32-fold improvement in S/N using HRMS over unit mass resolution, whereas propargite ($C_{19}H_{26}O_4S$) yielded a 20-fold higher LOD in HRMS than chlorpyrifos (as shown in Table 3). Most notably, they found that quantitative precision was adversely affected as mass resolution was increased, and that data acquisition settings required for reliable identifications and achieving highest detectability were "rather contradictory" [30]. They also showed that mass resolution was concentration dependent, and that pre-knowledge about the elemental composition of the analyte was very helpful in the identification of "unknowns." Furthermore, just as found in the case of standard 1200 GC–MS–MS, Cajka and Hajslova [30] encountered a similar situation with GC-HRMS using a TOF instrument in that the software reported falsely high S/N values for analyte peaks.

8.6. GC×GC–MS

Comprehensive two-dimensional GC×GC [29,36] represents another way to reduce matrix interferences through improved GC separation, by a factor of 5–10, and detectability enhancements of 1–50 [29]. We feel that some reports on detectability enhancements with GC×GC–MS are too optimistic since they consider only the gain in peak height of a broaden GC peak due to too slow temperature program and fail to properly account for the need to reduce signal averaging. Since GC×GC provides narrow GC peaks (e.g., 0.1 s), it is generally assumed that time of flight (TOF) MS is needed for GC×GC–MS [29,37]. However, we recently demonstrated that with flow modulation, we successfully performed GC×GC–MS analysis with quadrupole-based GC–SMB-MS, including real-world applications to diesel fuel and pesticides in agricultural matrices. GC×GC–SMB-MS is based on the use of flow modulation to broaden the GC×GC peaks to 0.2–0.25 s, which makes them amenable for the scan speed of quadrupole MS. Flow modulation requires system compatibility with 20 ml/min column flow rate without a loss of sensitivity, which is a unique feature of GC–MS with SMB. Our GC×GC–SMB-MS results are described in detail in another publication [38]. While the matrix interference reduction factor of 5–10 of GC×GC is somewhat lower than of MS–MS, GC×GC–MS excels in concurrent improvement of the instrumental detectability from narrower peaks, and unlike MS–MS, it provides universal, non-targeted sample analysis.

9. The 3-ion SIM method

In target analyses with quadrupole GC–MS, samples are often analyzed by SIM or full scan RSIM methods to obtain at least three ions for traditional identification purposes [22,39]. Initially, one may believe that the 3-ion method is less sensitive than SIM optimized for one ion by a factor of only 1.73 (square root of 3), which emerges from time sharing. However, the reality is that 3-ion SIM is less sensitive than 1-ion SIM typically by an order of magnitude (as shown in Table 3) considering the following facts: (a) the factor of 1.73 above; (b) the LOD depends on the weakest fragment among the three; (c) the weakest fragment is usually at lower *m/z* and has extended vacuum background and matrix interference noise; and (d) the need to comply with certain peak height ion ratio criteria for identification, which further increase the LOD in view of matrix interferences [22].

We investigated this subject and concluded that the use of two ions, one of which is the molecular ion, reduces the average degree of matrix interference by a factor of 90 over the 3-ion method [13]. In terms of sample identification, the molecular ion is clearly more important and provides a greater confidence level in sample identification than the two lower mass fragment ions in the 3-ion method. While the relative abundance of the molecular ion with the 1200-SMB can be about equal to that of the third ion in standard GC–MS, the noise level with the 1200-SMB is likely to be much lower (orders of magnitude) at the molecular ion than in standard GC–MS on the lowest mass third ion. Thus, in analyses that require more than 1-ion SIM in complex matrices, the detectability of the molecular ion in real-world sample compounds (not OFN) is particularly important.

In Fig. 12, we compare the analysis of permethrin performed with the 3-ion method using the 1200-SMB with the same analysis performed with a standard Agilent 5972 GC–MS at the USDA [40]. With the 1200-SMB, we were able to use the molecular ion (*m/z* = 390), and two fragments (*m/z* = 183 and 165), whereas with standard GC–MS, the molecular ion was absent and the

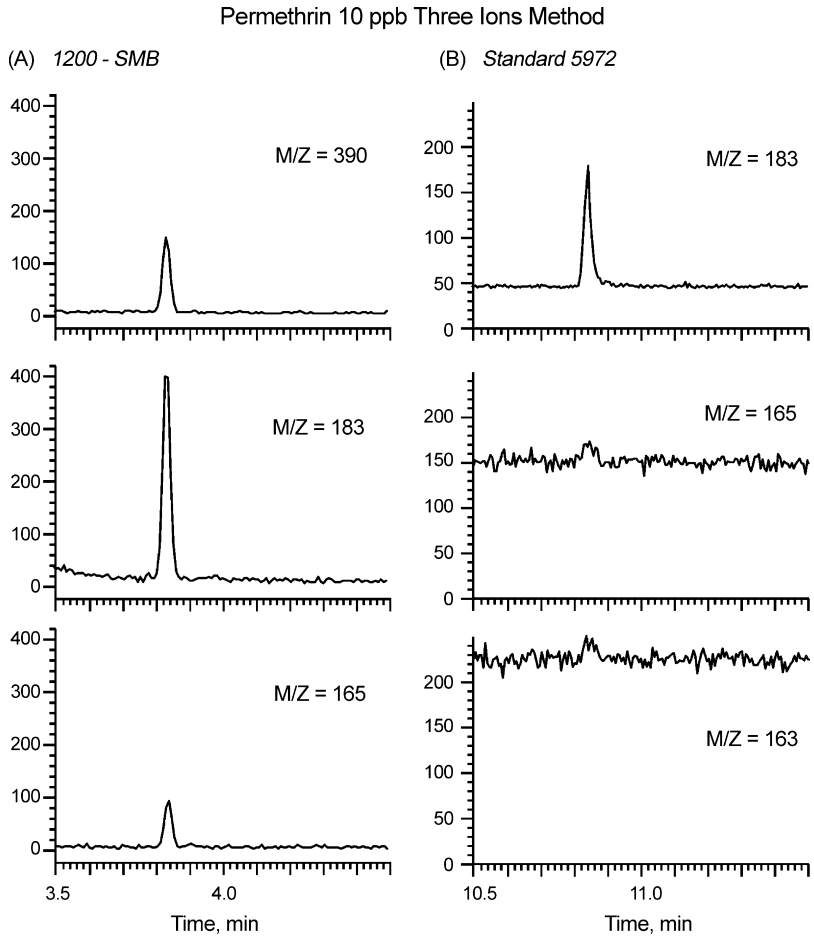

Fig. 12. Permethrin analysis with the 3-ion SIM method using the 1200-SMB (left) and Agilent 5972 MSD (right). The same 1 μl 10 ng/ml sample was injected in both systems. With the 1200-SMB, permethrin was analyzed using its molecular ion at $m/z = 390$ and major fragments at $m/z = 183$ and 165. With the standard 5972 MSD, the molecular ion was absent, thus permethrin was analyzed using $m/z = 183$, 165, and 163 fragments.

three highest mass fragments $m/z = 183$, 165, and 163 had to be used. With the 1200-SMB (left side of the figure), we obtained S/N of 32 for the 390 m/z molecular ion and S/N of 38 and 10 on the 183 and 165 m/z fragments, respectively (based on peak-to-peak noise). Our detectability with the molecular ion is therefore about 50 times better than of the standard GC–MS on the 163 m/z fragment ion. In addition, the 1200-SMB also shows 15 times higher S/N in the 165 m/z fragment due to lower noise. However, we assert that the low mass peaks of 165 and 163 m/z are not needed when the molecular ion is used to aid identification. Because detectability is limited by the weakest ion in the 3-ion method, we conclude that the 1200-SMB yields ≈50 times lower LOD for permethrin than standard GC–MS. Even if one insists on using three ions, the 1200-SMB also provides the $m/z = 392$ isotopomer of the molecular ion with S/N of 22, which is 36-fold better than what was found for the $m/z = 163$ with standard GC–MS.

Furthermore, matrix interference is expected to be reduced by a factor of 24 when the molecular ion and $m/z = 183$ fragment are used with the 1200-SMB in comparison with the 183, 165, and 163 m/z ions. The reasons for this entail: (a) a factor of 2 because two ions (165 and 163 m/z) are used compared with one ion (183 m/z) for the 1200-SMB; (b) the fact that the 183 m/z fragment is 6 times more abundant than the 165 or 163 m/z ions; and (c) the 183 m/z fragment is 20 m/z units higher than the other fragments, which leads to matrix interference reduction factor of about 2 [13]. Thus, accounting for all of these reasons, we anticipate a reduction of the matrix interference noise by a factor of 24. In practice, comparisons between Table 3 and Fig. 12 show permethrin LOD to be 48 pg by 3-ion SIM in matrix, 10 pg by 1-ion SIM in matrix, 1 pg by 1-ion SIM without matrix, and 1 pg by 2-ion SIM including the molecular ion in SMB-MS without matrix.

It should be emphasized that for a fair comparison the quality of both instruments needs to be documented. However, the 1200-SMB is not a commercial system and the 5972 system was used as it is routinely used at the USDA. Regardless, the results shown in Fig. 12 demonstrate that reasonably similar S/N was obtained for the 183 m/z main fragment of permethrin. The important and unbiased conclusion is that despite having similarly good S/N with both systems for the 183 m/z fragment, the 5972 MSD failed to analyze permethrin at 10 pg on-column level only because the 163 m/z fragment intensity is naturally low and its vacuum background was high, as commonly encountered in systems that are used for the analysis of samples in complex matrices. The superior performance of the 1200-SMB in the analysis of per-

methrin is attributed to the ability to use an enhanced molecular ion in combination with low vacuum background, instead of the 163 *m/z* fragment, as a second ion to the 183 *m/z* fragment.

10. Large volume injection to lower LODs

GC–MS is rarely sample amount limited, and typically the analysts care about the detectability only in the sense of having lower limit of concentration (LOC) for the entire analytical method (given in concentration of the analyte in the original sample) more so than LOD of the instrument (given in terms of amount of analyte injected). Thus, the lowest sample concentration that can be analyzed can be proportionally decreased by injecting larger sample volumes, unless matrix interferences are the limiting source of noise.

The subject of large volume injection (LVI) has been extensively described and discussed in refs. [41–44]. The 1200-SMB system offers three advantages with LVI: (a) its higher flow rate compatibility facilitates easier use of LVI; (b) its higher selectivity with the combination of enhanced molecular ion and/or MS–MS capability enables improved handling of the larger amount of matrix noise associated with LVI; and (c) the high column flow rate accommodates use of GC columns with increased capacity and robustness (lifetime), e.g., 0.53 mm i.d., which are more suitable for LVI.

In order to explore LVI with the 1200-SMB, we used a slightly modified Magni type LVI with the Varian 1177 standard split splitless injector [44]. Briefly, a large extract volume is injected into a hot injector while the column acts as a cryogenic pump to recondense the solvent and analytes at the column. We did not use a retention gap (pre-column) in our experiments. The reader is referred to Magni and Porzano [44] for a full account of this effective LVI method.

In Fig. 13, the success of this simple method is demonstrated with methylstearate, cholesterol, and $C_{32}H_{66}$, and as shown, the RSIM chromatograms of these compounds using their molecular ions are very similar between a 1 μl 10 μg/ml injection and 100 μl 0.1 μg/ml injection (same sample diluted 100-fold in methanol). Note that the signal intensity is presented in absolute counts, so the same signal height was obtained for the same injected amount, regardless of its concentration and injected volume. We do not claim any major achievement with this since there are a few other ways to perform LVI, but the LVI method that we used [44] is unique in its simplicity, flexibility and applicability to a standard split/splitless injector. It can also be used with any other injection volume in the 2–100 μl range. The Magni LVI method was developed for GC analysis (not standard GC–MS), and perhaps it is uniquely suitable to the GC–SMB-MS because it introduces the entire 100 μl solvent into the column and vacuum system. With the 1200-SMB this amount of solvent creates no problem because the jet separation is naturally stopped during the solvent elution and only ~1% of this solvent enters the ion source vacuum chamber. In conventional GC–MS, we are not sure if 100 μl solvent would adversely affect the ion source over the course of time. In addition, the rotary pump requires gas ballast operation with these large solvent volumes, which occurs as part of the process with the large helium flow rate of the 1200-SMB.

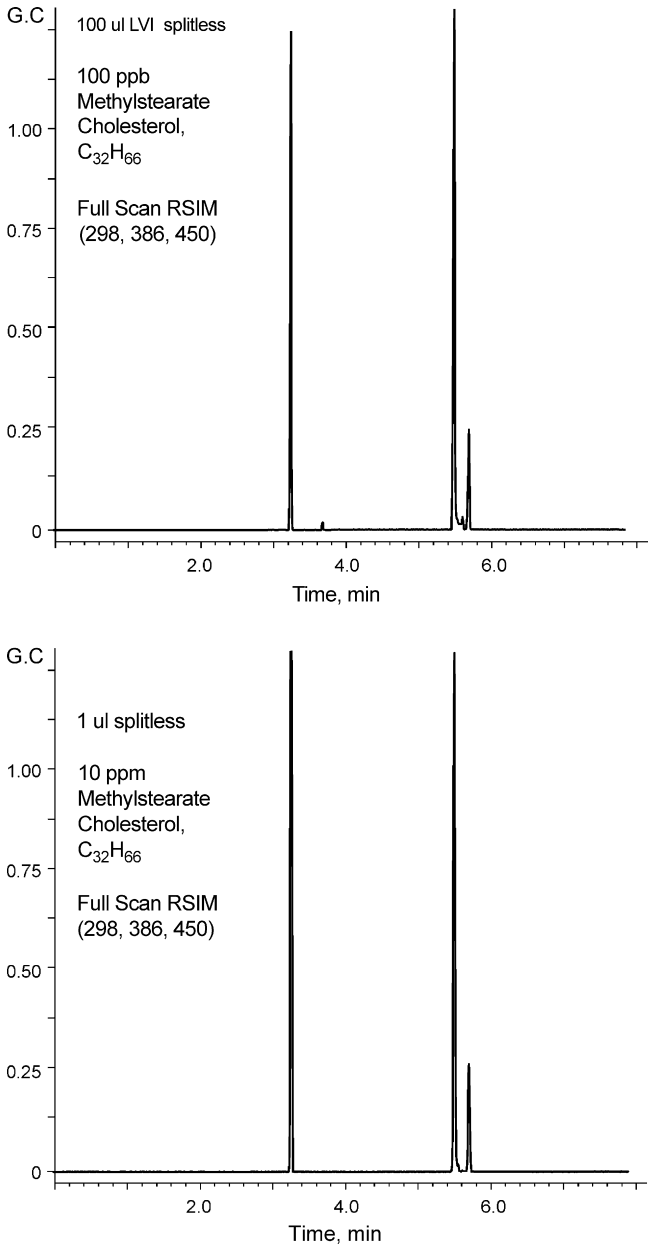

Fig. 13. LVI with the 1200-SMB using the standard 1177 injector. The injection of 1 μl 10 μg/ml solution (bottom) is compared with the injection of a 100 μl 100 ng/ml solution (top). The traces are RSIM on the molecular ions of methylstearate, cholesterol, and $C_{32}H_{66}$ at $m/z = 298.3$, 386.4, and 450.4, respectively.

In order to evaluate method LODs we prepared a solution of pyrene at 10 fg/μl (ppt) and injected 100 μl into the 1177 injector. The results appear in Fig. 14, which shows a nice pyrene chromatographic peak at 3.25 min, with a clean baseline despite the ultra-low sample concentration. The following achievements and features are concluded from the experiment: (a) the S/N obtained was ~500 in peak-to-peak terms (6746 RMS as calculated by the software), thus the demonstrated potential method concentration LOD (LOC) is 20 fg/ml (ppq), or 7 fg/ml using 5 RMS noise; (b) since the integrated pyrene amount is 1 pg, the instrumental LOD is 2 fg, the same value we

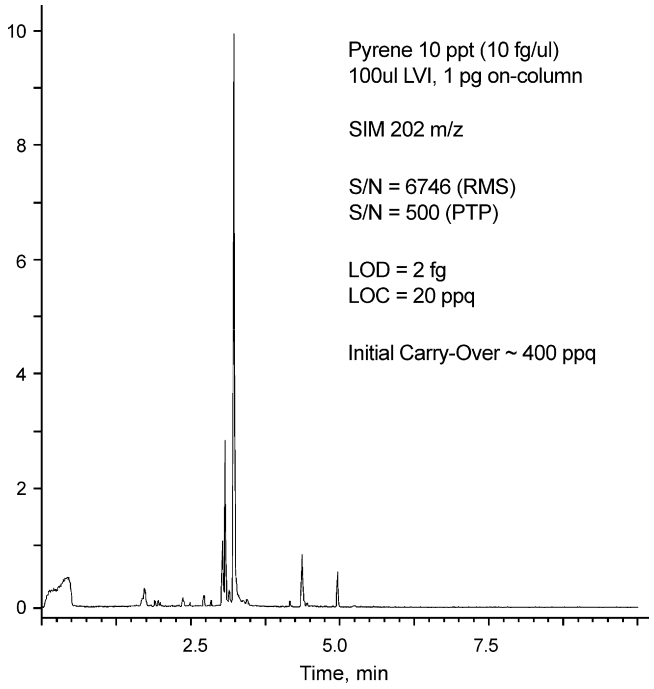

Fig. 14. LVI with the 1200-SMB and the demonstration of 20 fg/ml (ppq) concentrational LOD (LOC); 100 μl injection of 10 fg/μl pyrene concentration in methanol was injected using the Varian 1177 injector. Injector temperature was 250 °C while the GC oven initial temperature was 80 °C and programmed at 30 °C/min. Pyrene eluted as the largest peak at 3.25 min, using SIM at $m/z = 202$.

obtained for diazinon and only a little higher than for OFN; (c) several other peaks are observed due to solvent impurities which impose a much worse problem with LVI; and (d) clean chromatographic peaks arise despite the large volume injected (as in Fig. 13).

We do not claim that this method is useful without further improvements since we faced two major problems of short column lifetime and injector carry-over from previous injections. After about 10–15 injections, the signal started to reduce and column-induced peak tailing appeared. The short column lifetime could be due to the large solvent volume since these LVI injections are equivalent to 1000–1500 standard splitless injections in terms of column exposure to the solvent. However, we believe that the column deterioration emerged from injector impurities from the metal parts that were carried by the solvent to the column and not from the solvent itself. On the other hand, mega-bore columns (0.53 mm i.d.) can be used with SMB, as demonstrated previously [7–11], with their far superior robustness. The addition of a retention gap could also help. Carry-over was the major problem in obtaining ppq range LOD as blank injections gave pyrene peaks equivalent to 400 ppq, and the intensity of the pyrene peaks in the blanks grew in time. However, despite the above-mentioned problems, Fig. 14 demonstrates a record low GC–MS LOD in terms of concentration, which can become practical through the development of a proper LVI method and a clean lab environment.

Compatibility with LVI is clearly important for having good detectability. LVI with SMB can be particularly useful since the added matrix interference can be better handled via the superior 1200-SMB selectivity in view of its enhanced molecular ion, MS–MS, and better detectability particularly with difficult to analyze samples.

11. Conclusions

GC–MS users are often confronted by what we defined as the OFN gap, which is the large difference between the instrument LOD specifications for OFN (the best case scenario) and the much higher LOD in real-world applications. With the 1200-SMB, LODs in real-world applications are only somewhat higher than for OFN, for which the 1200-SMB achieved the record low LOD of 0.6 fg. The closure of the OFN gap with the 1200-SMB is attributed to the combination of: (a) enhanced molecular ion; (b) elimination of vacuum background noise; (c) elimination of mass independent noise in the 1200 MS in its Q2 and ion mirror; (d) elimination of ion source peak tailing and degradation; (e) lowering the sample elution temperatures; (f) significantly increased range of thermally labile and low volatility compounds that become amenable for analysis; (g) reduced column bleed; (h) reduced ghost peaks; (i) good compatibility with large volume injections; and (j) reduction of matrix interferences with the combination of enhanced molecular ion and MS–MS. As a result and in view of these 10 improved detectability parameters, excellent detectability is achieved with the 1200-SMB for a broad range of analytes, particularly for the most difficult analytes and even in complex matrices.

In this manuscript, we demonstrated the achievement of <1 fg OFN LOD milestone (SIM, $m/z = 272$) with the 1200-SMB. More importantly, we also demonstrated LOD of 2 fg for a more realistic compounds such as diazinon and pyrene, and 10 fg for underivatized testosterone which is not amenable for traditional GC–MS analysis. In comparison with standard GC–MS, we measured detectability enhancement factors of 24 for dimethoate, 30 for methylstearate, 50 for cholesterol, 50 for permethrin, >400 for methomyl, and >2000 for $C_{32}H_{66}$. In addition, naturally incurred testosterone was analyzed in alligator blood extracts without derivatization. In general, the harder the compound analysis, the greater is the 1200-SMB detectability gain, and the harsher the matrix, the lower is the relative 1200-SMB mass spectral noise.

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