The Multiple Benefits of Cold EI – Leading the Way to the Future of GC-MS

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Abstract

GC-MS is characterized by many performance aspects and most of them are not mentioned or discussed by the GC-MS vendors and/or by any user's paper. The purpose of this paper is to list 72 such performance parameters and discuss how Cold EI improves the vast majority of them. GC-MS with Cold EI is based on a GC and MS interface with a Supersonic Molecular Beam (SMB) and on electron ionization of sample compounds while they are vibrationally cold in the SMB (thus named Cold EI) in a contact-free fly-through ion source.

Cold EI Improves *all* the central GC-MS performance aspects, including: identification, mass spectral information, range of compounds that are amenable for analysis, sensitivity (detection limits), speed of analysis, and response uniformity.

Eight categories of GC-MS performances are listed below and in each of them we have several sub categories (given in the number in bracket):

A. Improved sample identification (13).
B. Extending the range of compounds amenable for GC-MS analysis (3).
C. Speed – faster GC-MS analysis (9).
D. Sensitivity (12).
E. Uniform, compound independent ion source response, quantitation and reproducibility (4).
F. Improved compatibility with GC-MS enhancement technologies (11).
G. Improved GC-MS maintenance, flexibility, ease of use and price (15).
H. Improved utilization of mass analyzer specifications (5).

Quoting Aristotle "The whole is greater than the sum of its parts", this combination of so many improvements (>60) creates a new and qualitatively superior technology that actually improves every type of analysis. While GC-MS with Cold EI enables new type of analyses and significantly improves challenging analyses, it does not impede on any simple method of analysis (compared with standard EI). Consequently, GC-MS with Cold EI is leading the way to the future of GC-MS.

Introduction

GC-MS vendors typically describe and characterize their systems using a small set of specifications that include octafluoronaphthalene (OFN) signal to noise ratio (SNR), mass range and scan speed. As a result, improvements in GC-MS are often focused on incremental increases of these few specifications. However, GC-MS is characterized by many additional features and operational parameters that contribute and characterize its performance, and their improvements can make a huge impact on the GC-MS analytical capabilities.
In this article we describe and show how Cold EI improves GC-MS not by one or few but by over 60 different aspects and performance parameters. We believe that the feature of significant extension of the range of compounds that are amenable for GC-MS analysis is the most important Cold EI benefit and the enhancement of the molecular ion abundance is its second most important benefit. However, following the famous statements "Quantity has a quality of its own" when these multiple Cold EI benefits are combined they are destined to be transformed into the next GC-MS revolution.

GC-MS with Cold EI – The Technology and System

GC-MS with Cold EI is based on a GC and MS interface with a Supersonic Molecular Beam (SMB) and on electron ionization of sample compounds while they are vibrationally cold in the SMB (thus named Cold EI). The ionization takes place in a contact-free fly-through ion source. An SMB is formed by the expansion of gas through a small shaped nozzle into a vacuum chamber. In this expansion, the combination of carrier gas, added helium make-up gas and sample molecules obtain the same final velocity, and as a result the sample compounds are accelerated to the helium velocity. This uniform velocity ensures slow intra-beam relative motion and collisions during the expansion, resulting in vibrational cooling of the sample compounds. As a result, SMB's are characterized by a few features that are highly advantageous for GC-MS including: a) Vibrational cooling of the sample compounds (enhanced molecular ions); b) High flow rate compatibility up to 100 ml/min (extended range); c) Compatibility with a contact-free fly-through EI ion source (many benefits).

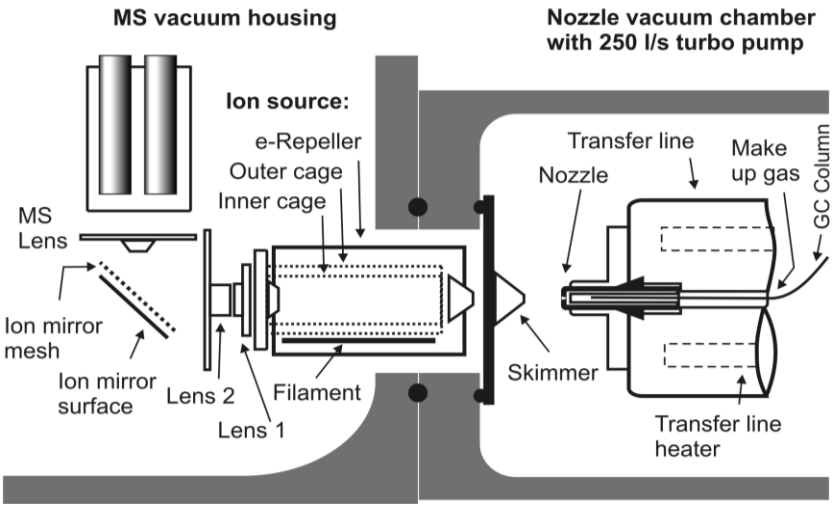

Figure 1. A schematic diagram of the Aviv Analytical 5977-SMB GC-MS with Cold EI. It is based on the Aviv Analytical conversion of Agilent 7890 GC + 5977 MSD into GC-MS with Cold EI.

We have explored the use of these unique properties of SMB for the early development of GC-MS with Cold EI over 28 years ago [1] and as reviewed in reference [2].

In Figure 1 a schematic diagram of GC-MS with Cold EI is shown with its supersonic molecular beam interface and fly-through ion source. Its various elements are indicated by their names. The basic modifications for the conversion of standard GC-MS instrument into GC-MS with Cold EI include:

a) The analytical column of a conventional GC with unrestricted column type, I.D., length and flow rate is connected to a supersonic nozzle via a heated transfer line and mixed with added helium make up gas (typically with flow rate of 50-60 ml/min).

b) Sampling to the MS vacuum system is in the form of a skimmed supersonic molecular beam, as the sample compounds expand with the added helium make-up gas from the supersonic nozzle into a separately (differentially) pumped nozzle vacuum chamber;

c) The electron ionization ion source is modified to allow unperturbed axial passage of the molecular beam (hence called "fly-through") with high (typically 8 mA) ionizing electron emission current;

d) A suitable 90 degrees ion mirror (or RF only quadrupole) is added to suppress mass spectral noise, keep the mass analyzer clean and for minimizing the added bench space.

What can be improved in GC-MS

In this article we list and describe 70 GC-MS improvements, categorized into eight separate sections, and show how GC-MS with Cold EI can provide these many benefits and improvements compared with GC-MS with standard EI via the unique properties of its interface and ion source.
One target of this paper is to that the list of possible GC-MS improvements is far greater than commonly perceived and most of these improved features are not even mentioned by any vendor or in any other type of publication or paper. Furthermore as shown below, many of these benefits affect several performance aspects and each is influenced by other features.

A. Improved Sample Identification

The coupling of mass spectrometry with gas chromatography is aimed at improved samples identification and quantification versus GC-FID and for serving as a major universal analytical analysis platform. While GC-MS excels in sample identification its performance can be significantly improved in a few ways. These improvements, as found in the Aviv Analytical 5977-SMB GC-MS with Cold EI can provide far greater confidence level in sample identification for significantly greater range of compounds:

1. **Enhanced identification of large and labile compounds.** The first and most important requirement of sample identification is that the sample will elute, and elute without column and/or ion source degradation. GC-MS with Cold EI enables the analysis of significantly extended range of compounds (in comparison with standard EI) including much bigger and more labile compounds. Thus, Cold EI not only improves sample identification but it also enables the analysis and identification of compounds which standard GC-MS fails to analyze [3].
2. **Enhanced molecular ions.** The molecular ion is the single most important mass spectral peak for successful sample identification. A major weakness of standard EI is that it produces molecular ions for only about 70% of the sample compounds and as the sample compound gets bigger its molecular ion relative abundance gets smaller. Cold EI

enhances the molecular ions abundance and for large compounds the degree of enhancement can be large [4]. The standard alternative to EI for the provision of molecular ions is chemical ionization (CI). However, while CI (or APCI) provides quasi molecular ions, its mass spectra are incompatible with the library search and identification, its response (ionization yield) is highly non uniform, certain compounds are not properly ionized with it, the closed CI ion source induces peak tailing and sample decomposition even more than standard EI ion source, it adds cost and requires venting for ion source replacement. Cold EI enhances the molecular ions which are provided for about 99% of the compounds combined with library identification compatibility, uniform ionization yield, extended range of compounds and without any of the CI downsides as above. The enhancement of molecular ion abundances also improves the sensitivity and selectivity of GC-MS analyses. In figure 2 the enhancement of the molecular ion in Cold EI is demonstrated for squalane (isoprenoid branched $C_{30}H_{62}$ hydrocarbon).

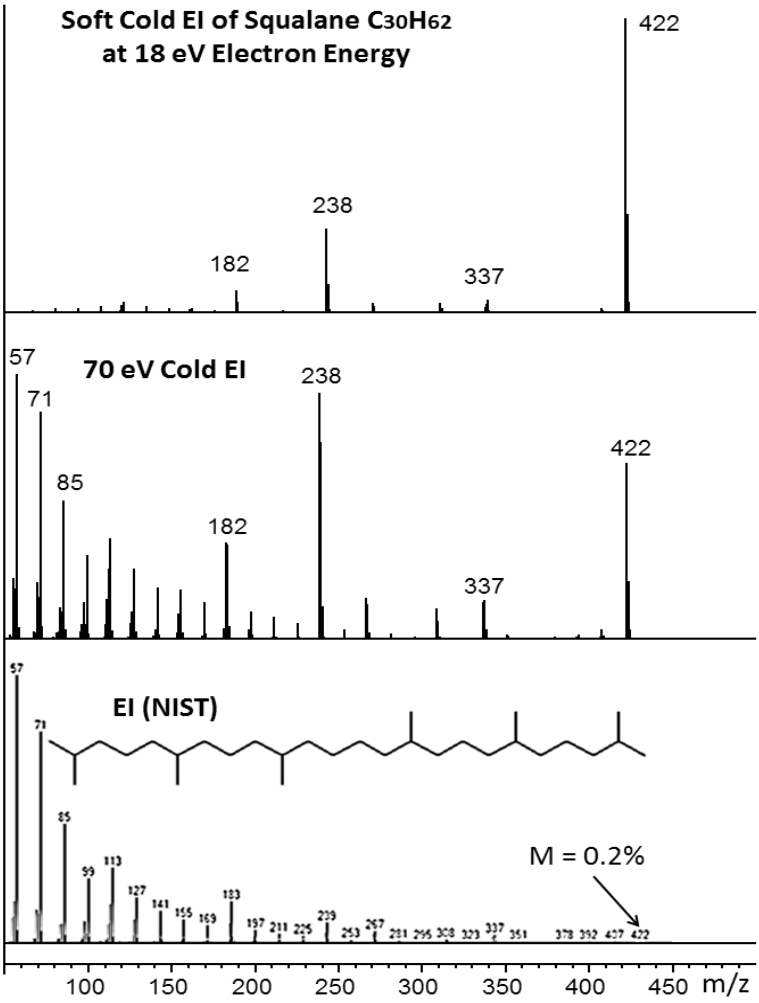

Figure 2. Molecular ion enhancement for squalane (A highly branched isoprenoid $C_{30}H_{60}$ hydrocarbon) as shown in the central trace compared with its NIST library standard EI-MS (bottom) and its low eV Cold EI (upper trace). Note the demonstration of largely enhanced molecular ion alongside the usual library searchable EI fragment ions. In addition, largely enhances isomer mass spectral information is exhibited. Another demonstrated benefit is that electron ionization can be a soft ionization method for certain compounds with low electron energies Cold EI that is approaching field ionization [5].

3. **Improved confidence in the identity of the molecular ions**. While the molecular ion can be found in standard EI mass spectra, often it is weak and cannot be trusted since it can be suspected to be an impurity, matrix ion, vacuum background or high mass sample fragment ion. In Cold EI the molecular ion can be trusted far more than in standard EI. This is in part due to the enhancement of the relative abundance of the molecular ion and in part due to the elimination of vacuum background of ions with

masses above the molecular ion. Thus, in Cold EI the highest mass spectral peak can be assumed with high confidence level to be the molecular ion since it is usually fairly abundant and there is nothing in the mass spectrum above it. This benefit is particularly useful in the identification of unknown compounds that are not included in the EI-MS libraries.

4. **Improved library search and identification capabilities.** One of the most important advantages of GC-MS and its standard EI ion source is that EI mass spectra of about 270,000 compounds that are available in the NIST library enable easy to obtain, fast and automated identification. Cold EI mass spectra, with their enhanced molecular ions, retain the lower mass fragment ions and are thus fully compatible with library based sample identification. Furthermore, while the enhancement of the molecular ions reduces the library (such as NIST) matching factors it often improves the library identification probabilities since the latter largely reduces the matching to competing candidates, as described in details and with computer simulations in [6]. Additionally, the availability of the molecular ions provides a good "manual" confirmation (or rejection) of the library identification via the identity of the molecular ion. ***Accordingly, Cold EI is the only "soft" Ionization method that is compatible with NIST library identification***.

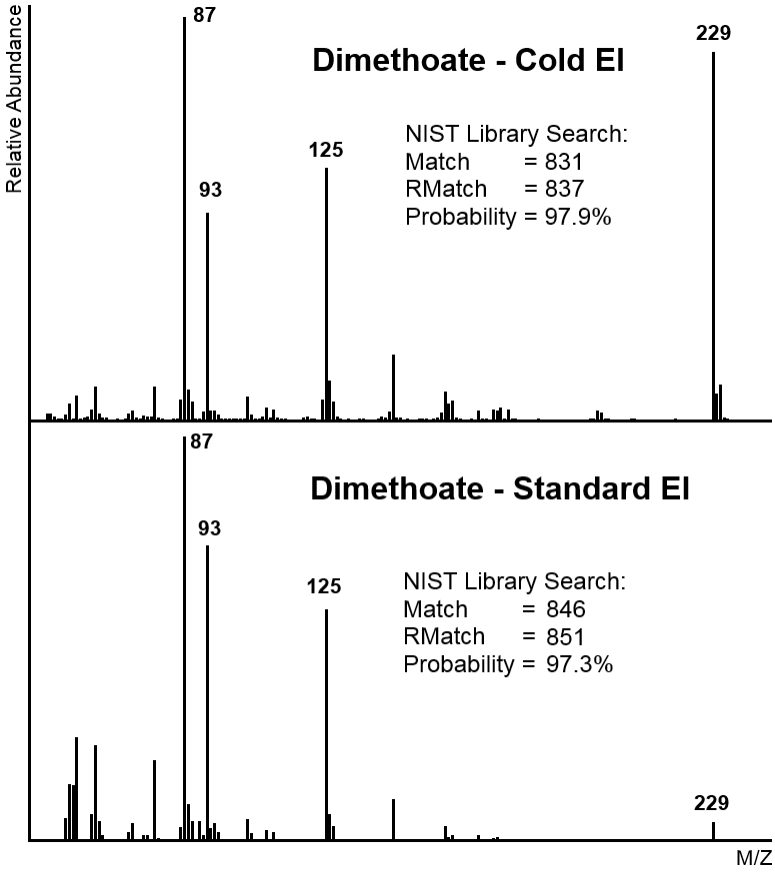

Figure 3. Cold EI and NIST library based identification. As shown, Cold EI provides "lower fit but better hit" for the pesticide dimethoate and similarly for most other compounds. While the matching factor of the sample compound is reduced in Cold EI the matching factors of competing compounds are reduced even more. This is due to the fact that molecular ions are the most characteristic sample MS ions.
As a result, the NIST library identification probability of 97.3% in standard EI is improved in Cold EI to 97.9%.

5. **Provision of accurate isotope distributions of the molecular ions.** Cold EI provides abundant molecular ions with accurate isotope distributions without any ion source related self chemical ionization or vacuum background interference, and thereby with unperturbed accurate isotope distributions. Isotope Abundance Analysis (IAA) provides important information (as used with isotope ratio MS and for isotope labeling experiments) and enables the elucidation of elemental formula of an analyte [7].

6. **Provision of elemental formulae with unit resolution quadrupole MS**. Clearly, it is highly desirable to be able to obtain elemental formula from low cost unit resolution quadrupole based GC-MS systems particularly for the many (most) compounds that are not in the EI-MS libraries. We developed the Tal-Aviv Molecule Identifier (TAMI) software that works well with standard EI MS and excels with Cold EI MS. TAMI automatically converts the molecular ion group of isotopomers and the measured molecular ion mass which it can improve its mass accuracy into elemental formulas and automatically (zero clicks) confirms or rejects the identification results of the NIST library. As a result, Cold EI with TAMI provides the ideal and ultimate sample identification technology for low cost unit resolution quadrupole MS. For further information on the TAMI software please visit its Aviv Analytical website. http://www.avivanalytical.com/Isotope-Abundance.aspx

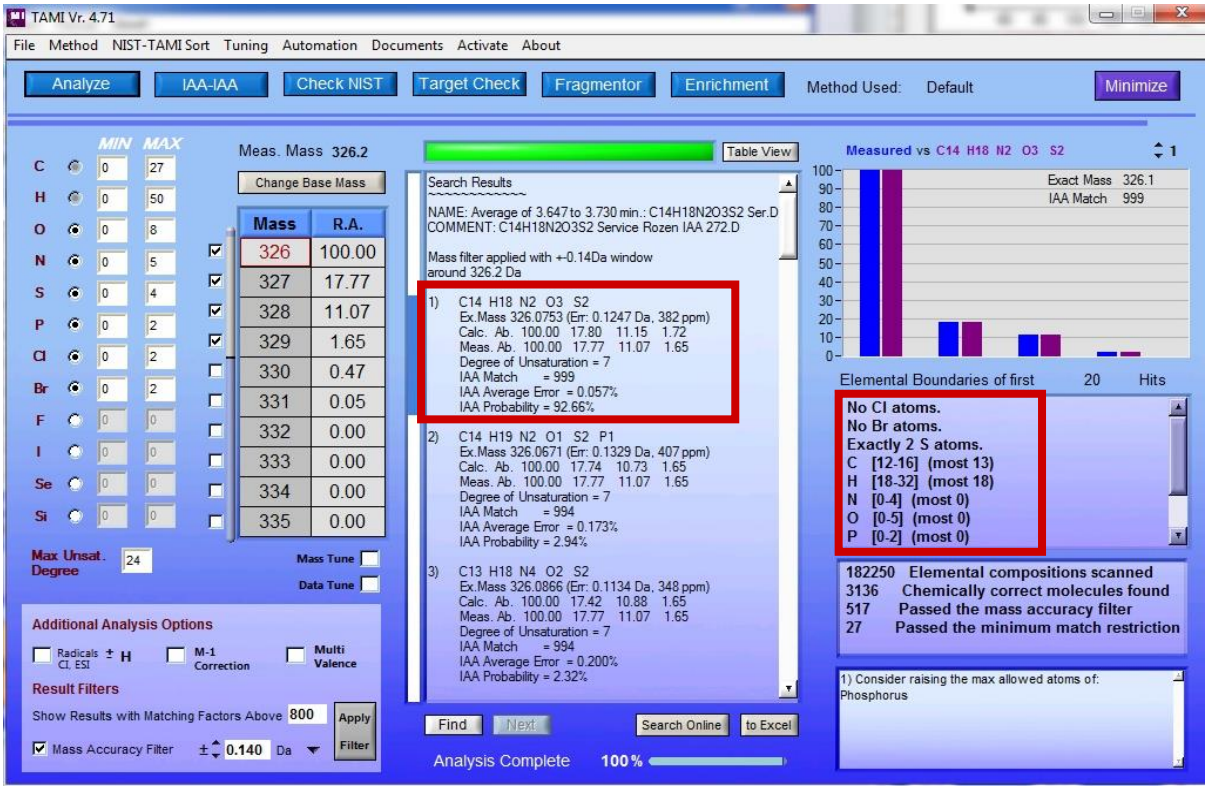

Figure 4. TAMI software screenshot that shows the provision of elemental formula for an unknown synthetic organic compound $C_{14}H_{18}N_2O_3S_2$ from the 5975-SMB GC-MS with Cold EI data which was later confirmed by NMR. While standard EI failed in the analysis of this organic synthetic compound Cold EI succeeded and provided its molecular ion for its TAMI analysis plus determined the chemical reaction yield of this compound. Note also that TAMI with Cold EI provides, unlike any high resolution MS, also elemental boundaries (as shown in red at the right side of Figure 4) thus we know that this compound does not have Cl or Br atoms while it must have 2 sulfur atoms and between 12 and 16 carbon atoms.

7. **Increased isomer and structural MS information**. Cold EI further enhances the MS peaks of high mass fragments and as a result provides greater structural and isomer MS information than standard EI as demonstrated in Figure 2 and described in [8].
8. **Fragmentation tunability.** Tunable fragmentation enables the elucidation of order of fragmentation hence helps to obtain the molecular structure. In Cold EI in view of the elimination of intra-ion thermal energy, the electron energy is the only remaining

parameter that controls the intra ion energy, and the reduction of the electron energy allows better control and reduction of the degree of molecular ion fragmentation. While Cold EI electron energy does not provide full fragmentation control (in many cases the effect is small), it still clearly improves on this feature in comparison with standard EI.

9. **Classical EI mass spectra with the fly-through ion source.** While Cold EI outperforms standard EI, some users feel uncomfortable without the ability to obtain "classical EI" mass spectra. Such mass spectra can be beneficial if the need arises to distinguish between certain isomers via the library. Cold EI, unlike CI, enables method based (software controlled) easy and fast switching to "standard" EI for the provision of classical EI mass spectra. This conversion is simply performed via lowering the Cold EI combined column and make-up gas flow rate to 5 ml/min without any need to physically replace an ion source or open the vacuum chamber [9, 10]. Furthermore, one can use slightly higher make-up gas flow rate and obtain slight enhancement of the molecular ions which results in having the best matching factors with the NIST library since the NIST search algorithm emphasizes the molecular ions. In addition, while classical EI with the fly-through ion source and SMB (which we name Classical EI-SMB) provides classical EI mass spectra, it also exhibit a few other benefits over typical standard EI ion sources such as in the provision of uniform response and extended range of compounds that can be analyzed as described in [10]
10. **Cluster Chemical Ionization (CCI)**. Cluster CI mode of ionization is provided with the fly-through ion source via a simple method based addition of methanol vapor from a methanol vial into the Helium make up gas [11, 12]. Cluster CI further enhances the molecular ions and results in having protonated molecular ions and methanol satellite (M+32 and/or M+33) ions that serve to ensure the identification of the molecular ions masses. This is possible as fragments do not show such satellite peaks since the cluster is the first to fragment. Cluster CI was found by us as useful to provide molecular ions in the few rare cases that Cold EI does not provide them such as for the PETN explosive compound or small alcohols such as octanol.
11. **Deuterium exchange.** Cold EI enables a unique mode of intra-nozzle deuterium exchange that helps in OH, NH and SH groups identification for further improved structural and isomeric elucidation [11]. Its fly-through ion source prevents back exchanges thus it is much better than standard EI for this application. In addition, one can inject sample compounds in deuterated solvent (such as CD_3OD) and have partial deuterium exchange in view of partial back exchange at the GC liner and column while intra ion source back exchange is fully eliminated.
12. **Isomer distribution analysis.** As often the case, new instrument concepts leads to the development of new and powerful methods of analysis. The enhancement of the molecular ions obtained with Cold EI and the unique availability of these important ions for branched hydrocarbons and other types of isomers led to the development of isomer distribution (abundance) analysis. This type of analysis is an important new method for fuel and hydrocarbon mixture characterization via their origin and refinery unique isomer distribution patterns. We note that the isomeric content and distribution affects all the chemical and physical properties of fuels and oils including: combustion efficiency, octane number, flash point, viscosity, lubrication properties, solubility and solvation power, boiling points and melting points, and thus, their determination is very important for these industries. More information on this important new method is available in reference [8]. It further serves in entomology research and other applications [13].

13. **Achieving the lowest limits of identification.** Achieving low limit of identification is far more demanding than low limit of detection since identification requires: a) Detection of the sample compound as a peak in the total ion mass chromatogram (TIC); b) Having appropriate separation of the sample compound from other compounds; c) Having good ratio of peak to vacuum background and column bleed interferences; d) Having a trustworthy molecular ion; e) Having informative mass spectral fragment ions for good library based identification and/or structural information, particularly if the compound is not in the library. We demonstrated that GC-MS with Cold EI exhibits much lower limits of identification than the Agilent GC-MS with HES ion source [14] due to the elimination of vacuum background noise, elimination of ion source related peak tailing and having enhanced molecular ions.

B. Extending the Range of Compounds Amenable for GC-MS Analysis

The recognized and well-known Achilles heel of GC-MS is its limited range of volatile and thermally stable compounds that are amenable for analysis. This limitation emerges from GC injector, column and ion source induced sample degradation and/or lack of sufficient vaporization. The limited range of compounds amenable for GC-MS analysis is further exacerbated by the fact that the relative abundance of the molecular ion is reduced with the sample compound size (mass) and for large compounds even if they elute, without exhibiting trustworthy molecular ions they cannot be properly analyzed. In CI this limitation is even greater than in EI in view of its closed ion source structure. GC-MS with Cold EI enables significant extension of the range of compounds amenable for analysis via the use of short columns with high column flow rates in combination with full elimination of intra-ion-source degradation [2, 3, 15]. GC-MS with Cold EI provides the ultimate range of compounds amenable for GC-MS analysis and effectively bridges the gap between standard GC-MS and LC-MS. The key parameter for this unique capability is the use of very high column flow rates which in combination with the use of shorter columns leads to significantly lower elution temperatures (up to 200°C), while the provision of the enhanced molecular ions compensates for the traded GC separation. High column flow rate further reduces intra injector liner degradation (lower elution temperature from the liner to the column and reduced liner residence time) and intra-ion-source sample dissociation is inherently avoided due to the Cold EI fly-through ion source geometry. Details of this important feature are provided in ref [3]. Improved GC-MS range of compounds amenable for analysis can be separated into three groups as below:

14. **Significantly extended range of thermally labile compounds that are amenable for analysis.** The significantly lower elution temperatures from the GC column in combination with the use of temperature programmable injector with high injection flow rates enables a major increase in the range of thermally labile compounds that can be analyzed. The latter is complemented and supplemented by the Cold EI enhanced molecular ions that proves the elution of intact thermally labile sample compounds. In fact, GC-MS with Cold EI is equivalent and even superior to LC-MS with APCI or APPI in its range of thermally labile compounds that are amenable for analysis. In APCI and/or APPI the sample is thermally vaporized at a very hot vaporization (liner like) oven (400-500ºC) where the sample stays for a short time while in GC-MS with Cold EI the sample is vaporized at much lower injector temperatures and spends much longer time at the column at much lower temperatures. Since the effect of thermal degradation exponentially depends on the temperature, Cold EI can be gentler to thermally labile compounds than APCI/APPI and excels in the analysis of carbamate pesticides such as Aldicarb or explosives such as TATP.

15. **Significantly extended range of low volatility compounds that are amenable for analysis.** The significantly lower elution temperatures from the GC short column with high flow rates enables a major increase in the range of low volatility compounds that can be analyzed. We found that we can approximately double the molecular weight and size limit of compounds that can be analyzed in comparison with standard GC-MS to about 1200 amu for non-polar compounds [15] and 800 amu for polar compounds. In fact, GC-MS with Cold EI is equivalent in its range of compounds to LC-MS with APCI but unlike Cold EI these LC-MS ion sources fail to ionize non-polar compounds such as hydrocarbons.
16. **Significantly extended range of polar compounds that are amenable for analysis.** The elimination of ion source tailing and degradation on its metallic surfaces enables the analysis of many polar compounds without their derivatization. Examples include the analysis of free fatty acids [16], amides [14] and compounds with free OH without derivatization (such as cholesterol) and at low levels [17]. These compounds are found in many analyzed sample mixtures but are ignored since they are not observed at low levels with standard EI.

In Figure 5 we show a cartoon that demonstrates our perception of the average total ion count (TIC) and molecular ion signal dependence on the sample compound mass obtained with Cold EI and with standard EI. From this figure one can calculate the average sensitivity gain of Cold EI with the 5977-SMB GC-MS with Cold EI versus sample mass. Note that as the sample mass is increased the molecular ion abundance in standard EI is reduced. While on the average 30% of the NIST compounds do not exhibit molecular ions, for compounds above mass 400 about 50% do not have a molecular ion. In contrast, in Cold EI the molecular ion abundances are about mass independent. In addition, the total ion count signal is reduced with mass and its reduction due to ion source peak tailing and degradation is much stronger in standard EI than in Cold EI.

In Figure 6 we demonstrate the extended range of compounds amenable for analysis with Cold EI in the analysis of triglycerides (TAGs) in interesterified palm oil [18]. Note the availability of useful molecular ions plus informative high mass fragments to all the triglycerides while each TAG peak is characterized by many isomers and degrees of unsaturation. As shown in the TIC mass chromatogram the peaks are symmetric, tailing free and with high ratio of signal to column bleed. In addition, we could easily identified and differentiate diglycerides that eluted among the smaller triglycerides via the fact that the Cold EI MS of triglycerides had an abundant molecular ion and fragment ions with a loss of fatty acid mass while diglycerides were characterized by a major fragment ion with a loss of water (18 amu). A list of over 50 "extended range" applications of GC-MS with Cold EI is available on request.

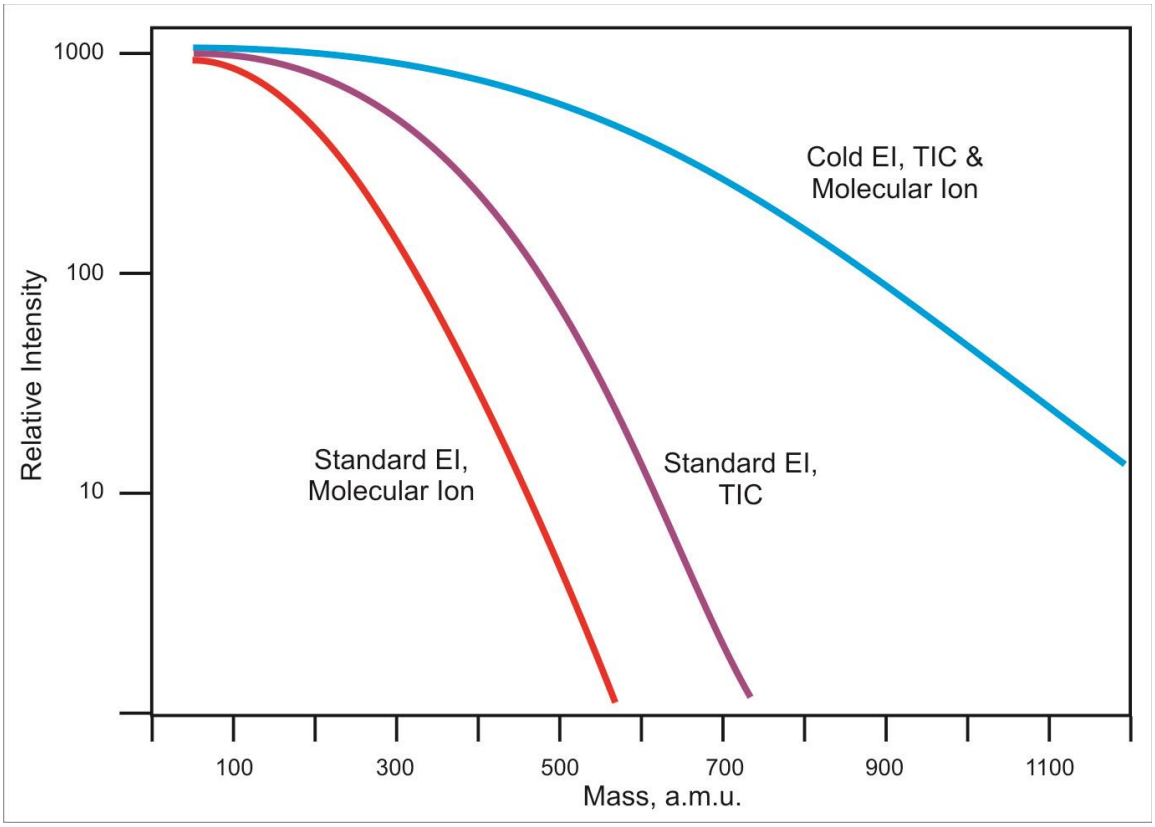

Figure 5. A cartoon that demonstrates our perception of the average total ion count (TIC) and molecular ion signal dependence on the sample compound mass. Accordingly, it also demonstrates our expected average sensitivity gain of Cold EI with the 5977-SMB GC-MS with Cold EI versus sample mass, which at some point is transformed into extended range of compounds amenable for analysis.

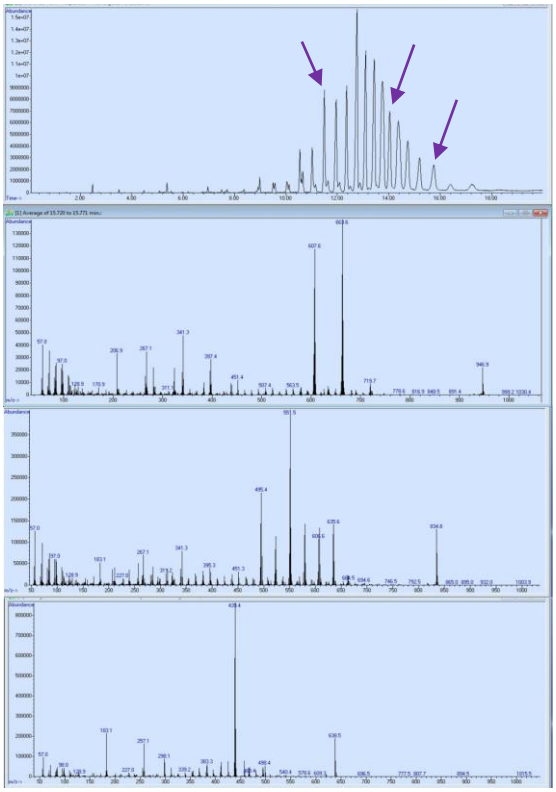

Figure 6. Triglycerides in palm oil analysis by GC-MS with Cold EI, as an example of extended range of compounds amenable for GC-MS analysis. Total ion count mass chromatogram (upper) and three Cold EI mass spectra of triglycerides in interesterified palm oil are shown. The Cold EI mass spectra were obtained on the arrows indicated TIC mass chromatogram peaks. Note the availability of useful molecular ions plus informative high mass fragments. The TIC peaks are symmetric, tailing free and with high ratio of signal to column bleed. Note that each TAG peak is characterized by many isomers and degrees of unsaturation. The Cold EI mass spectra of triglycerides has an abundant molecular ion and fragment ions with a loss of fatty acid mass while diglycerides were characterized by a major fragment ion with a loss of water (18 amu).

C. Speed – Faster GC-MS analysis

The reduction of analysis time is an obvious needed GC-MS improvement. While there are few ways to reduce the analysis time they all involve trade-offs and thus fast GC-MS is an art of finding the optimal trade-off and Cold EI provides great trade-off flexibility. GC-MS with Cold EI enables the highest capability fast GC-MS, from the reduction or elimination of sample preparation to the final fast analysis results. Basically, fast GC-MS is achieved based on the trade-off of GC resolution (separation) for speed of analysis. The sacrificed GC separation is compensated with enhanced separation (selectivity) power of the MS and/or MS-MS via the enhancement of the molecular ion. A few GC-MS aspects can be improved for achieving faster analysis as discussed below:

17. **Faster splitless injection.** In standard GC-MS the splitless injection takes at least additional three minutes as compared to split injection. The injection takes a minute plus another minute or more for the temperature of the GC oven to rise to start the separation process, plus another minute or more for cooling down from that initial separation temperature to about 50ºC which is needed for the splitless injection to achieve cryo-focusing. While split injection can be much faster via avoiding the above wasted time problems, it often involves with unacceptable signal loss. GC-MS with Cold EI uniquely enables fast splitless injections. With Cold EI, very high injection column flow rates can be used, such as 30-60 ml/min, hence the splitless injection takes a second or two, similarly to a split injection, and it can be performed at higher initial GC oven temperature, thereby saving a few precious minutes from the injection time, temperature program rise and cooling down time.
18. **Freedom in choice of the most suitable column.** Standard GC-MS typically uses 30 m columns with 0.25 mm I.D. and 1 ml/min column flow rate which restricts the analysis time. The use of a shorter 0.1 mm I.D. microbore column can shorten the analysis time by a factor of 2.5 while retaining the separation but with a trade-off of on-column sample amount, column loadability (sample capacity) and column lifetime. Fast GC-MS with Cold EI is characterized by an unrestricted selection of column type, I.D., length and flow rate and in this aspect GC-MS with Cold EI is similar to LC-MS in which the user can select any column length, diameter and particle size for optimal trade-off of separation for speed of analysis. Note that with the reduction of the column length and/or increase in its flow rate, every reduction by a factor of two of the GC separation peak capacity enables four times faster analysis. Furthermore, while the use of short columns facilitates faster analysis, the use of high column flow rates is preferable since it can be controlled without any hardware change and time programmed with sub second response time (unlike GC oven temperature). As a result, GC-MS with Cold EI enables the optimization of the GC-MS analysis method for achieving the shortest analysis time while delivering the needed results via an optimized selection of column I.D., length and flow rate.
19. **Fast column cleaning.** With Cold EI one can program the column flow rate to speed-up and reduce the time needed for column cleaning from late eluters to avoid ghost peaks in the next analyses. Accordingly, Cold EI with its flow program can shorten the analysis via reducing a few minutes at the end of GC-MS runs.
20. **Flow programming.** GC-MS with Cold EI can uniquely add column flow programming capability during the analysis with no need to re-tune the instrument and uniquely significantly reduce the analysis time this way. We demonstrated for heroin (Figure 7) and cocaine the use of flow programming from 1 to 32 ml/min to obtain under 2 minutes full chromatography time and under 3 minutes full analysis cycle time including cooling back and ready for next injection. [19] with standard GC oven.

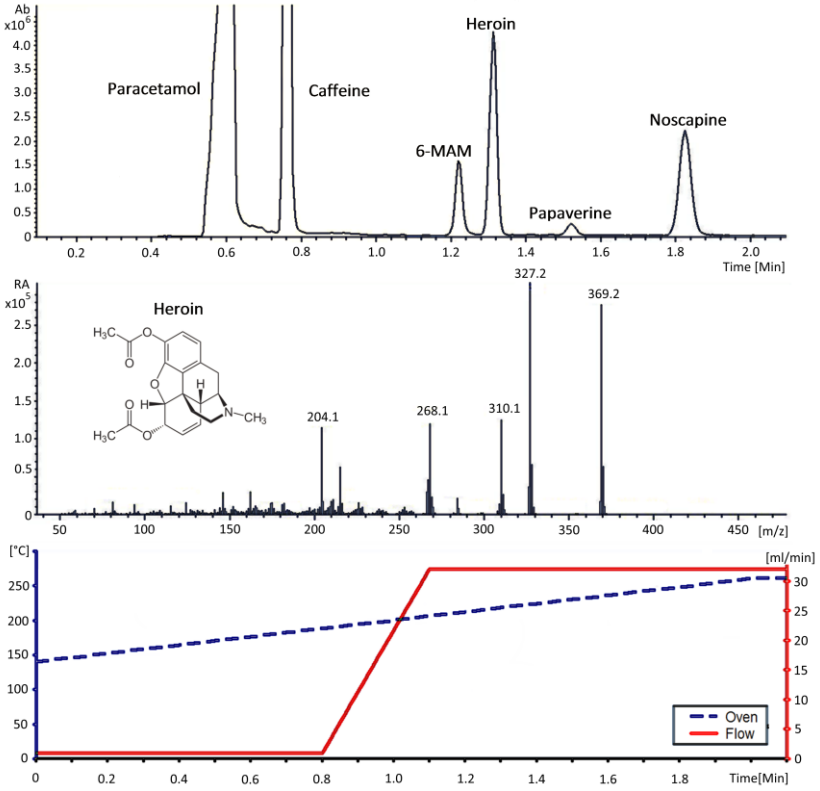

Figure 7. Flow program based fast GC-MS with Cold EI analysis of heroin in its street drug powder. The obtained full scan total ion mass chromatogram is shown at the upper trace while the middle trace shows the obtained Cold EI mass spectrum of heroin. The names of the separated compounds are given near or above the various peaks. The bottom portion of the figure shows the outline of temperature and flow program parameters as a function of time.

21. **Ultra-fast ion source response time.** While the fast GC-MS literature is full of discussions conducted by proponents of time of flight MS about the need for fast scan speed, no one mentions the equally or even more important role of fast ion source response time and elimination of ion source peak tailing. In the analysis of semi-volatile and/or polar compounds GC-MS peak tailing originates in the ion source due to its slow response time and hampers the GC separation, particularly in fast GC-MS and/or GCxGC-MS. Cold EI on the other hand is characterized by ultra-fast sub-millisecond ion source response time and full elimination of any ion source tailing due to its design where the supersonic beam passes through it with no wall contact. A full discussion of this aspect can be viewed in our post on peak tailing [20].
22. **Compatibility with the scanning speed of quadrupole MS**. Quadrupole is the most widely used mass analyzer in GC-MS. However, it has a somewhat limited scan speed which today is in the 10,000-20,000 amu/s range by most vendors (limited to below 10000 amu/s if one wishes to retain isotope fidelity). Achieving fast GC-MS with Cold EI through the use of high column flow rates and short widebore (0.53 mm I.D.) or standard narrowbore (0.25 mm ID) columns provide fast analysis with peak width in the order of 0.3 - 0.5s (or more) that are fully compatible with the scan speed of quadrupole MS that can exceed 20 Hz scan rate for 50-550 amu mass scan range.
23. **Extract-free dirty sample introduction without lengthy sample preparation**. While the chromatographic separation could be fast, for truly fast analysis the time devoted for sample handling and preparation must be reduced as well. The use of the ChromatoProbe (Agilent name Thermal Separation Probe) for sample introduction for its intra GC injector thermal desorption facilitates extract-free sample introduction for broad range of solid and sludge samples and thereby reduces the time devoted for sample handling and clean up. The use of the ChromatoProbe with GC-MS with Cold EI benefits from a higher possible injector flow rate for easier and softer sample thermal desorption with higher recoveries. More information on the ChromatoProbe can be

found in reference [21] and in its product website [22]. In addition, using short columns and high column flow rates combined with flow programming at the end of the analysis guarantees that every compound that is introduced from the injector into the column is eluted. Consequently, faster and simpler forms of sample preparation such as immersion and injection can be practiced as described in our post on the analysis of synthetic cannabis [23].

24. **Improved compatibility with Low Thermal Mass Ultra-Fast GC**. When the sample contains several compounds with broad volatility range, fast analysis requires using a low thermal mass (LTM) fast GC that can provide fast temperature programming rate. We developed an LTM Fast GC which enables full analysis cycle time of under one minute with temperature programming rate of up to 2000°C/min and cooling back time of under 20 seconds [24]. GC-MS with Cold EI is fully compatible with the LTM Fast GC use of short columns and high column flow rates. High column flow rates are highly desirable for the rapid temperature programmed Fast GC-MS in order to elute the late eluting compounds before the high temperature plateau end of analysis where the peaks can be broadened and delicate samples can decompose.
25. **Shortest sample handling time - Open Probe Fast GC-MS**. Our Open Probe Fast GC-MS (Agilent name QuickProbe) is the latest and most advanced development in the field of real time analysis. In addition to real time analysis, and unlike any other real time analysis technique, it also provides separation and library identification. The Open Probe [25] further reduces the time devoted for sample collection and preparation. Further information on the Open Probe Fast GC-MS including its comparison with DART and DESI can be found in our two papers [26, 27]. The main benefits of Open Probe Fast GC-MS with Cold EI over other real time analysis techniques are that Cold EI provides enhanced molecular ions, extended range of sample compounds amenable for analysis and uniformed response for improved analysis.

D. Sensitivity

GC-MS sensitivity is one of its prime specifications and although not very important for qualitative applications it serves for many users as a symbol of the system quality. However, the OFN specification that is used to measure GC-MS sensitivity is inappropriately provided by all GC-MS vendors and does not properly represents the GC-MS sensitivity (or LOD) as discussed in reference [28] and in our blog post on sensitivity specification [29]. The subject of GC-MS sensitivity is more involved than commonly perceived and GC-MS sensitivity depends on several parameters and performance features that can be improved as explained in the points listed in this section. As discussed below, Cold EI improves the GC-MS sensitivity (lowers its LOD), hence the 5977-SMB GC-MS with Cold EI is the most sensitive GC-MS and the harder it is to analyze a compound the greater is the Cold EI gain in sensitivity.

26. **Ion source signal strength.** Standard EI ion source is operated with 30-100 µA emission current and with few spiral paths of electron motion that are magnetically confined. This ion source provides good ionization efficiency which nowadays approaches 0.1% at the ion detector and even higher with the recent Agilent HES and Thermo AEI ion sources that are based on axial magnetic fields. The Cold EI fly-through ion source is operated typically with 8 mA emission current and with about three cycles for each ionizing electron thus it has high ionization yield despite the x10 faster motion velocity of sample compounds in the SMB and absence of multiple sample passages at the ionization volume as in standard EI. However, further SMB skimmer collimation losses (x4-5) and ion mirror losses (x3) reduce the Cold EI overall ion signal which is similar to that of standard EI.

27. **Ion source noise – the elimination of vacuum background noise.** Standard EI ion sources have two major noise sources**:** vacuum background and helium metastable (neutral) related mass independent noise. While the mass independent noise was suppressed in standard EI via the use of improved ion detector and ion optics designs (such as the Agilent triple axis ion detector), vacuum background still remains and is the most prominent source of noise. While OFN specifications are obtained in a mass spectral region that has minimal vacuum background noise, with a new and clean vacuum system and with electro-polished ion source surfaces (that are scratched and become rough after the first ion source cleaning), in real world applications vacuum background could be high and severely limit the obtained S/N. In Cold EI, vacuum background is filtered out in the dual-cage fly-through ion source. This filtration is enabled due to the fact that sample compounds in the supersonic molecular beam have a few eV directional kinetic energy, while vacuum background species have non-directional <0.1 eV kinetic energy at the ion source. Thus, the combination of directional SMB compound motion and a small electrostatic repulsion potential in one of the fly-through ion source lenses fully eliminates vacuum background noise. This improved reduction of noise level improves the obtained S/N in Cold EI which is superior to any standard EI ion source and lead to noise free OFN mass chromatograms which allows OFN 1 pg RSIM S/N specification of any desirable value including $>10^{+6}$.
28. **Elimination of mass independent noise.** Helium related mass independent noise is one type of noise that exists in all GC-MS systems. It emerges from the co-formation of metastable helium atoms during the electron ionization process, and these metastable atoms can either directly ionize molecules upon collisions or generate free electrons upon their scattering from surfaces that can directly contribute to noise or ionize sample and/or vacuum background compounds after the mass analyzer. Curved pre quadruple ion optics and improved ion detectors with multiple axis ion paths significantly reduced this type of mass independent noise. However, the effect of such mass independent noise reduction on the overall sensitivity is limited since the most important type of noise caused by vacuum background still remained. In Cold EI vacuum background is fully eliminated as explained above, making the elimination of mass independent noise a relatively more important task. In the 5977-SMB GC-MS with Cold EI a 90º ion mirror is used in order to obtain further significant suppression of mass independent noise, and as a result its mass independent noise count rate is very low, below 1 ions/s.
29. **Reduced column bleed and ghost peaks noise.** Column bleed with its multiple mass peaks of m/z = 73, 147, 207, 281, 355, 429, 503 (and their isotopomers) etc. is a known major source of noise that hampers the detection and identification of low volatility compounds. In addition, ghost peaks which belong to previous runs (sample compounds that entered the column and did not elute in the previous few runs) further complicate and increase the apparent column bleed noise. Cold EI enables the use of shorter columns with higher column flow rates and column flow rate programming at the end of the run. As a result, the sample compounds elute at significantly lower temperatures [3] so that column bleed and ghost peaks noise can be eliminated altogether. The lower elution temperatures and flow programming column cleaning significantly reduce any ghost peak. In addition, the use of transfer-line temperature programming further reduces PDMS related bleed noise to a minimum and thus Cold EI is characterized by exceptionally low noise particularly for large compounds.
30. **Enhanced molecular ions for improved sensitivity.** The molecular ion is by far the most informative and selective ion. Thus, for the sensitive and selective detection of any compound it is advised to monitor it via its molecular ion either in SIM or full scan RSIM modes (or both). Thus, the enhancement of the molecular ion directly improves

the sensitivity (lower LOD). In Cold EI the molecular ion is enhanced while keeping the total ion count. The degree of enhancement can be small or modest for small and rigid compounds such as benzene or OFN but large, more than three orders of magnitude, for large aliphatic compounds as demonstrated and discussed in [2, 4]. As a result, Cold EI particularly excels in the sensitive detection of large and difficult to analyze compounds.

31. **Reduced ion source peak tailing.** One of the adverse GC-MS EI ion source effects that limits its sensitivity is ion source peak tailing. A small chromatographic peak tail hides, like an iceberg, a significant loss of TIC signal [20]. Ion source peak tailing can be reduced via increased EI ion source temperature but as the ion source temperature is increased the molecular ions are exponentially reduced for many classes of compounds and intra-ion-source degradation is promoted. Ion source peak tailing reduces the chromatographic separation, lowers the sample signal, increases its MS noise and increases the signal RSD. With Cold EI, ion source related peak tailing is fully eliminated in view of the use of a contact-free fly-through ion source. Thus, as the sample size and/or polarity are increased and its volatility is reduced the gain in S/N with Cold EI is significantly increased. The tailing-free ultra fast ion source response time is provided with Cold EI, regardless of the sample's volatility. For more information on the topic of peak tailing and how to eliminate it please read our post on this topic [20].

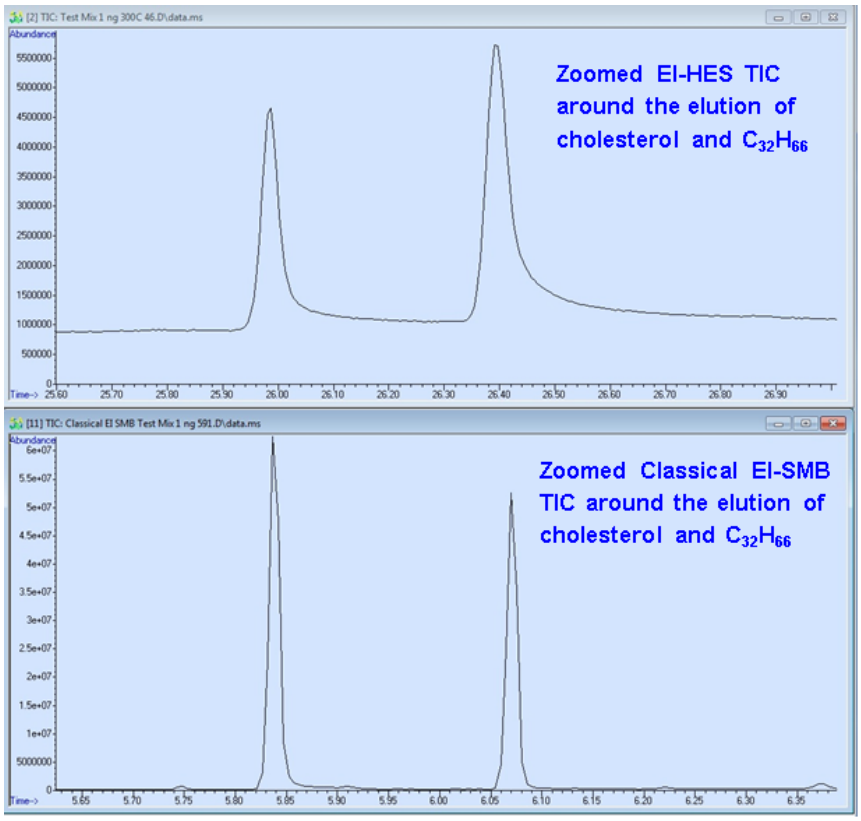

Figure 8. An example of peak tailing elimination in GC-MS with Cold EI in its Classical EI-SMB mode (bottom trace) compared with GC-MS with the HES ion source at 300ºC (upper trace) for cholesterol and n-$C_{32}H_{66}$. Note also the narrower and symmetric peaks in Cold EI that also have much greater ratio of peaks to baseline.

32. **Improved ion source inertness for increased range of thermally labile compounds that are amenable for analysis.** Regardless of the selection of ion source materials and the various inertness claims made by vendors, standard EI ion sources are active due to unavoidable contact of sample compounds with metal surfaces, and since all metals act as catalysts to degrade many types of organic compounds. The use of electrically conductive materials such as metals is essential at the ion source to create optimal

electric fields in it. As a result, standard EI ion sources are not inert and induce sample decomposition for many compounds. Cold EI is an inherently inert ion source since it uses a contact-free fly-through ion source configuration. No sample contact inherently means no sample degradation at the ion source. This feature of ultimate ion source inertness leads to enhanced sensitivity particularly when it is most needed in the analysis of sample compounds that are difficult to analyze such as thermally labile compounds.

33. **Improved compatibility with large volume injections.** Large volume injection (LVI) is a known technique that can improve the concentration sensitivity and provide lower detected concentration. It is usually performed via the injection of larger than the standard 1 µL sample volume with a temperature programmable GC injector. However, at some point the injection of further larger volume leads to increased column, liner and ion source contamination while the increased signal is offset by similarly increased matrix noise. With Cold EI the matrix interference on the molecular ion is minimal [30, 31] thus Cold EI can further benefit from LVI. Furthermore, with the use of short column and flow programming at the end of the run, practically everything that entered from the liner into the column elutes, thereby keeping the column clean. Cold EI enables another convenient mode of "Larger Volume Injection" that utilizes standard splitless injections with very high pulsed splitless flow rates (such as 30-60 ml/min) that are possible with short columns. In such injections conditions, the sample is vaporized and swept into the column at a combination of high pressure (lower vaporize sample volume) and at a rate of >1 µL per second hence injection volumes in the range of 2-10 µL can be employed with standard injectors without injector temperature programming and its related loss of volatile sample compounds.
34. **Improved selectivity for reduced matrix interference**. Matrix interference is the most important source of noise that limits the LOD in the analysis of samples in complex matrices such as drugs in urine or blood and/or pesticides in agricultural products. However, we found that matrix interference is exponentially reduced with mass by a factor of ~20 every 100 amu [30] and as discussed in our blog posts discussing this topic [31, 32]. Thus, the enhancement of the molecular ions in Cold EI enables the detection of the sample compounds in complex matrices with significantly less matrix interference hence with much lower LOD. ***In fact, the detection of pesticides in agricultural products via the molecular ions in Cold EI is as selective and sensitive as their detection by MS-MS on a parent fragment ion in standard EI while enabling full scan for universal pesticide analysis.*** When MS-MS is employed on the molecular ion the selectivity is further increased and both first and third quad resolution can be opened for higher signal and for the best MS-MS sensitivity.
35. **Lower level impurities analysis.** Another type of "sensitivity" which is not often discussed regards low level impurities analysis in a given material and the most known such requirement is in the analysis of impurities in active pharmaceutical ingredients (API) or in simple terms the analysis of impurities in drugs. This topic is separately discussed with examples in our posts on drug impurities analysis [33, 34]. While initially such analysis seems simple as the required detection limits are typically 0.1%, of the API in fact it is a challenging application. The challenge begins with the fact that uniform response is needed in order to know the concentration of the impurity without knowing its identity and without requiring a lengthy procedure of impurity sample calibration. Two factors are most detrimental for this type of analysis; a) Drugs are typically polar compounds and so are most of their impurities so that even if they are thermally stable they tend to saturate the column at <10 ng amount via the formation of peak fronting. When the GC peak starts to exhibit fronting it means that any additional on-column sample amount will not increase the peak height but it will only broaden the

peak via its increased front hence hamper the analysis of nearly co-eluting impurities. We found that most drug impurities are either isomers or homologous and related compounds that elute near the main API compound in GC-MS. b) Ion source related peak tailing reduces the total ion count signal for polar drug-like compounds, particularly at low levels [17]. When the column capacity is below 10 ng and the TIC signal to noise ratio is below 100 (peak to peak) for 1 ng impurities, at the 0.1% concentration level they are barely or not detected and certainly cannot be identifies. With Cold EI the peak tailing losses are eliminated thus it provides better TIC sensitivity. In addition, high column flow rates increases the column capacity which depends on the separation plate film volume that linearly increases with the flow rate. As a result, Cold EI excels in the analysis of impurities and as demonstrated in our posts on this topic it can detect 0.1% impurities with S/N >100 (peak to peak noise) [33, 34].

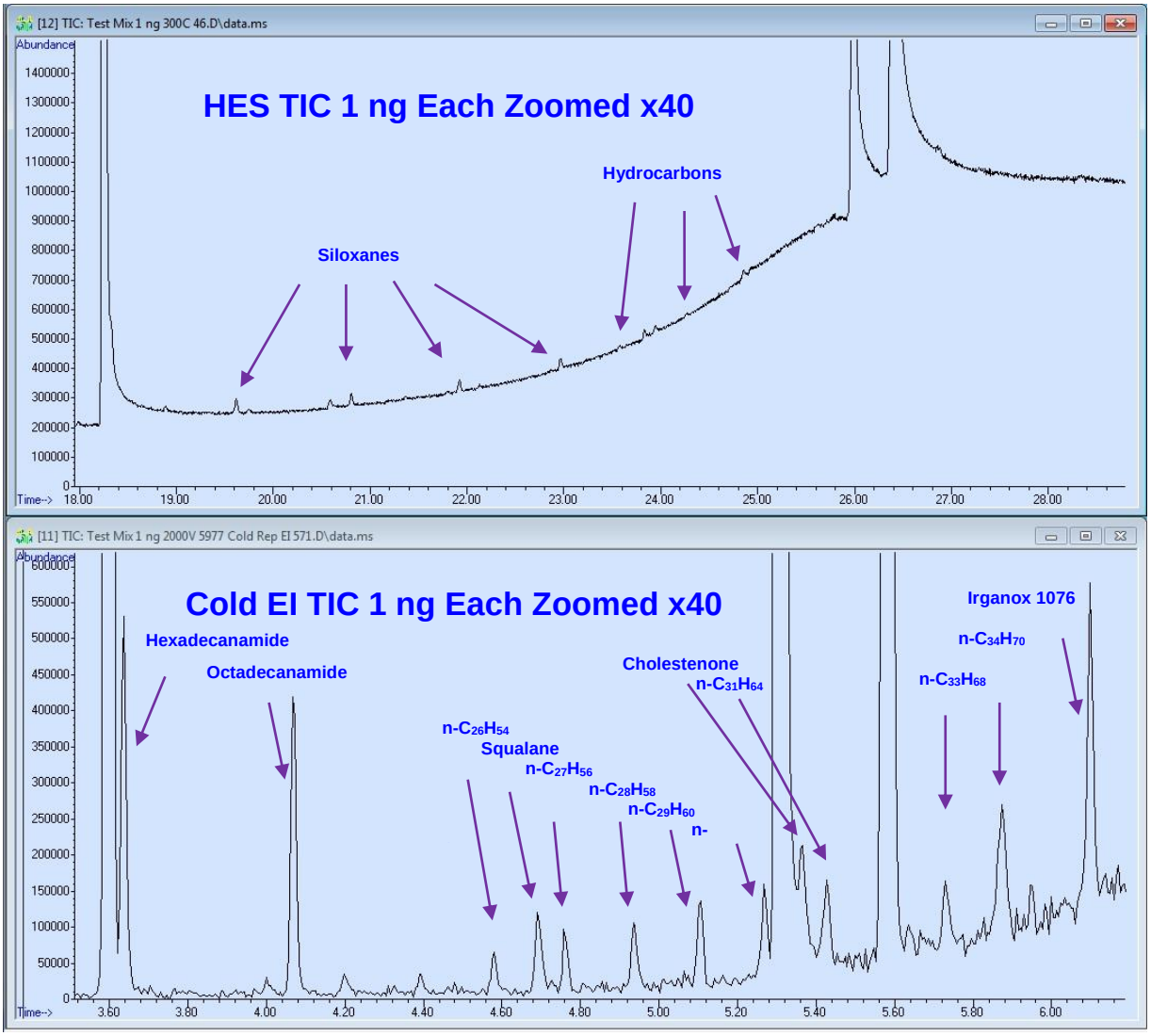

Figure 9. Low level impurities analysis – Standard EI versus Cold EI. Zoom on mass chromatograms in the analysis of a test mixture containing hexadecane (n-$C_{16}H_{34}$), methylstearate, cholesterol and n-$C_{32}H_{66}$ (in order of elution) with on-column amounts of 1 ng each. The upper mass chromatogram was obtained by the Agilent 5977B MS with HES ion source while the bottom mass chromatogram was obtained by the Aviv Analytical 5977-SMB GC-MS with Cold EI. The mass chromatograms were amplified 40 times and the time axis

starts just before the elution of methylstearate and ends after the elution of cholesterol and n-$C_{32}H_{66}$. The names and arrows indicate the identified impurities in the Cold EI mass spectrum. Note the much better total ion mass chromatogram signal to noise ratio in Cold EI for the mixture impurities that were all analyzed by Cold EI and failed to be analyzed by standard EI. In addition the amides (hexadecanamide and octadecanamide) were not observed at all in standard EI while they were easily detected and identified by Cold EI.

36. **More representative sensitivity specifications.** Clearly, the use of OFN full scan RSIM specification is inappropriate and misleading as discussed in our post on OFN specifications [29, 35]. GC-MS sensitivity should be characterized by several specifications given for a range of compounds that include OFN (as the easiest to analyze compound) plus a few other compounds that are gradually more difficult to analyze. Accordingly, the test mixture of the Aviv Analytical 5977-SMB GC-MS with Cold EI includes OFN, Hexadecane (n-$C_{16}H_{34}$), Methylstearate, Cholesterol and n-$C_{32}H_{66}$. Representative specifications can include: a) RSIM S/N on all the mixture compounds; b) SIM S/N; c) Total ion count (TIC) S/N for all the test mixture compounds, which in our opinion is the most representative sensitivity specification as it includes both signal and square root of the background noise level; e) TIC over baseline ratios which relate to the identification limits in which Cold EI excels as shown in Figure 9. While Cold EI exhibits similar SIM signal to noise ratio as standard EI for OFN, its signal to noise ratio is much higher for harder to analyze compounds as shown in Figure 10, and for example for Cholesterol and n-$C_{32}H_{66}$ is can be over 600 better for Cold EI. The superior Cold EI sensitivity for cholesterol as demonstrated in Figure 10 emerges from: a) The cholesterol TIC signal is reduces x16 times versus early eluting compounds in 1 ng on-column amount due to ion source peak tailing and at 10 pg this signal reduction factor should be much higher; b) The molecular ion was enhanced x25 times in its abundance in Cold EI; b) The cholesterol noise was much higher in standard EI due to its higher temperature elution and thus exhibited extended ghost peaks and column bleed noise. We similarly performed this same comparison experiment with the Agilent HES most advanced ion source and it performed worse for these compounds than the inert ion source due to lower abundance of the molecular ions and much greater noise. Thus, Cold EI exhibited >1000 times superior S/N for cholesterol and >5000 times for n-$C_{32}H_{66}$ than standard EI in its most advanced HES configuration. We note that the above sensitivity comparison is performed with SIM on the molecular ions since in full scan RSIM there is no (zero) noise in Cold EI [35] for all the five compounds in the test mixture thus Cold EI is vastly superior to standard EI but no number can be given.
37. **Reduced limit of identification.** Often detection must include trustworthy identification. While the subject of what is meant by identification is not trivial it is easy to agree that improved identification relates to improved total ion count signal to noise ratio plus having more abundant sample characteristic ions such as the molecular ions and high mass fragment ions. Cold EI enhances the molecular ions and high mass fragments and provides improved total ion count signal to noise ratio that can be > 1000/ng. In Figure 9 and [14] we demonstrate how Cold EI excels in having lower limit of identification in comparison with standard EI.

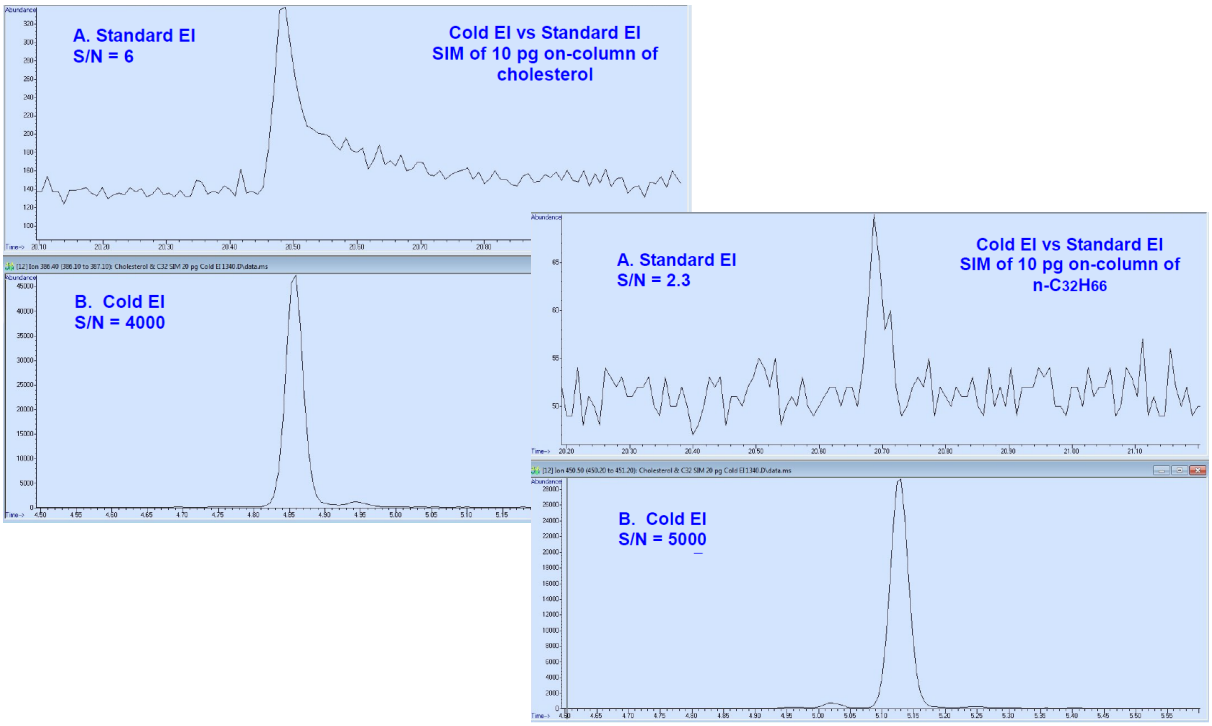

Figure 10. Sensitivity comparison of 5975-SMB GC-MS with Cold EI versus 5977 with Standard EI with 10 pg on-column in SIM mode for Cholesterol and n-$C_{32}H_{66}$ on their molecular ions. Cold EI exhibits >600 times superior S/N for cholesterol and >2000 times for n-$C_{32}H_{66}$ than standard EI (also better chromatography, faster analysis and much better identification when full scan is used).

E. Uniform Compound Independent Ion Source Response, Quantitation and Reproducibility

Uniform compound independent response is a highly needed feature which is absent in LC-UV and/or LC-MS. GC with FID is well known to be a semi-quantitative analytical tool while GC-MS is similar to GC-FID for volatile sample compounds but its response uniformity is eroded for semi volatile compounds due to ion source related peak tailing. Uniform response provides the ability to know the relative amount of any unknown compound or impurity without the lengthy, tedious and expensive procedure of its separation, identification, synthesis and the performance of compound specific calibration curve. Thus, uniform response is of particular importance in the areas of drug impurities analysis and for the elucidation of chemical reaction yields and its optimization as discussed in [36].

38. **Uniform, compound independent ion source response.** The electron ionization cross section approximately depends on the number of electrons in the sample compounds hence on their molecular weights thus on the sample weight. As a result, for volatile compounds the EI TIC mass chromatograms provide uniform compound-independent peak area responses, similar to those of GC-FID. However, as the sample compound becomes bigger, more polar and/or less volatile, ion source peak tailing becomes more and more pronounced and consequently the standard EI ion source response uniformity is eroded and lost. Cold EI provides uniform response regardless the sample volatility and provides it for about doubled mass range of compounds amenable for analysis (compared with standard EI). This feature is translated into a unique Cold EI capability - the provision of chemical reaction yields, something that is missing in ESI LC-MS or

standard GC-MS systems. We consider this benefit of Cold EI as one of its most important features as it uniquely enables the measurement of impurities abundances even if they are active compounds such as those with OH, NH or SH. One example is the analysis of Cannabis for its cannabinoid compounds. In standard GC-MS the peak of cannabidiol is much smaller compared to THC from its correct value due to increased reactivity at low levels with the ion source surface and only Cold EI can provide the correct relative amounts of such cannabis compounds. Figure 11 clearly demonstrates the response uniformity of Cold EI while in standard EI with the Agilent HES ion source the response declines with the sample size, elution temperature (time) and polarity of the sample compounds even at 300ºC ion source temperature. Accordingly, while cholesterol total ion count signal is the biggest in Cold EI it is 16 times lower than that of n-hexadecane in the HES TIC.

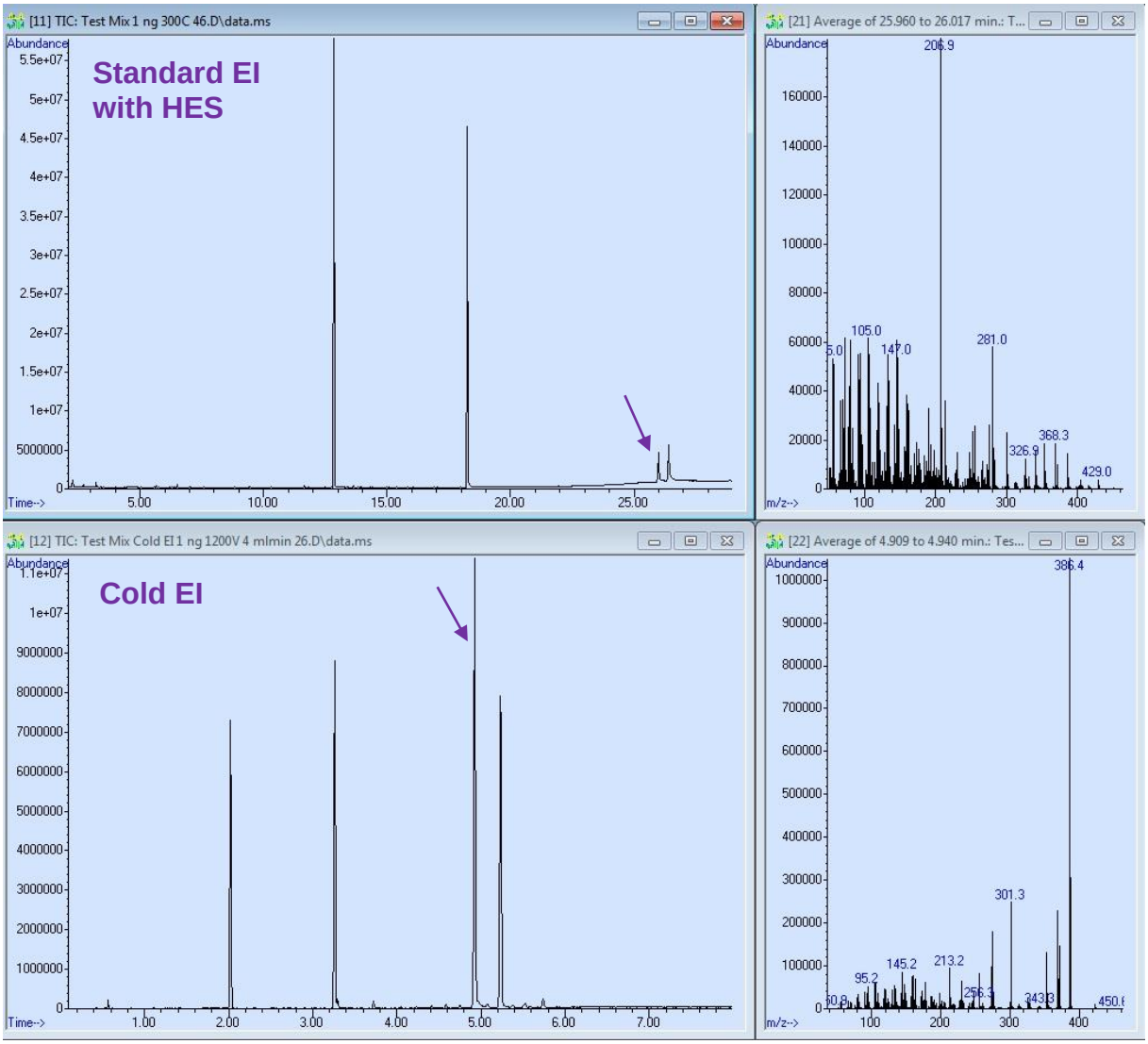

Figure 11. Response uniformity in Cold EI and its absence in standard EI. A comparison of a test mixture analysis results that were achieved with Agilent 5977B with high efficiency ion source (HES) at 300ºC (upper trace) and the same 5977B system operated with Cold EI ion source. The mixture includes 10 pg OFN and 1 ng on-column each n-hexadecane, methylstearate, cholesterol and n-$C_{32}H_{66}$. At the right side are the corresponding mass spectra of cholesterol with HES (upper) and Cold EI (bottom), both without background subtraction.

In addition to the demonstration of the Cold EI far superior response uniformity, Figure 11 also demonstrates several additional Cold EI benefits including: a) Enhanced molecular ion in cholesterol (25 times); b) Clean Cold EI mass spectrum with the elimination of vacuum background and column bleed interference; c) About five times faster Cold EI analysis; d) Much longer time available for heavier compounds elution in Cold EI (extended range); e) Elimination of ion source related peak tailing that is observed with the HES even without zooming while it is absent in Cold EI; f) Better TIC sensitivity and lower limits of identification is demonstrated via the observation of several small TIC peaks in Cold EI in the elution time range 3.4-6 min which are not observed in standard EI (zoomed and can be better seen in Figure 9)

39. **Quantitation.** Very good quantitation capability is considerred as one of the most important features of GC-MS yet it is rarely discussed for real life compounds. The electron ionization process in both standard EI and Cold EI exhibits inherent linearity as the ionization is of free electrons of compounds in vacuum. The slope of the linearity plots for simple compounds such as OFN is close to 1.000 and limited mostly by our ability to properly dilute the OFN sample and GC injection RSD. Additional minor issue is leakage current at the filment base which could be coated by partial conductive layer and which can change with time. In standard EI it can range from 1 up to few µA which can be significant compared to typical 36 µA emission current while this leakage current is much smaller compared to the Cold EI typical 8 mA emission current. However, the most important obstacle for proper quantitation is ion source related peak tailing which significantly adversely affect the linearity at low levels hence impede on quantitation in standard EI. In Cold EI on the other hand, linear response is observed all the way down to low levels [17].
40. **Linear Dynamic Range (LDR).** Usually the LDR is limited by the ion detector range but for large and polar compounds it becomes smaller in standard EI. Cold EI improves the LDR particularly for large and polar compounds in both sides of the response curve. At the low on-column amount due to its contact-free fly-through design Cold EI exhibits linear response while standard EI behaves in a non-linear way [17] due to ion source related peak tailing and interaction with its metallic surface and thus have higher LOD. On the other side of the response range the GC column has limited sample capacity which is reduced for large and polar compounds and thus GC peak fronting and distortion is exhibited. With Cold EI the use of high column flow rate proportionally increases the sample capacity hence the LDR plus 0.53 mm I.D. can also be used with its much higher sample capacity. Thus, Cold EI exhibits bigger LDR than standard EI for large and polar compounds.
41. **Reproducibility.** As discussed in the quantitation section the standard EI ion source peak tailing and degradation results in non-linear response and this non-linearity depends on the ion source temperature, cleanliness and history thus results in high RSD and poor reproducibility for polar and labile compounds plus those with free OH or NH which as a result require time consuming derivatization. Cold EI exhibits superior reproducibility for these difficult to analyze compounds. For example, the Cold EI mass chromatogram shown in Figure 11 is highly reproducible while the HES mass chromatogram visibly changes every analysis in the ratio of n-hexadecane to the last to elute cholesterol and n-$C_{32}H_{66}$ and in the ratio of peaks of cholesterol to n-$C_{32}H_{66}$.

F. Improved Compatibility with GC-MS Enhancement Technologies

Enhancement technologies are important add-on techniques, devices and software that serve to further improve the performance of GC-MS. Usually they are offered as options to the basic GC-MS systems but some can be integrated or bundled such as the NIST library.

Known enhancements are the CI ion source, MS Probe, bigger Turbo molecular pump or split pump, MS libraries, auto-samplers and thermal desorption units. Clearly, improved compatibility with enhancement technologies can further improve GC-MS performance and its range of applications as discussed below for GC-MS with Cold EI.

42. **MS Probe, ChromatoProbe and intra injector thermal desorption devices.** GC-MS can benefit from having MS probe in having faster analysis and in the analysis of labile compounds yet with the trade-off of GC separation. One type of such MS probe is the ChromatoProbe sample introduction device that provides fast probe sampling and instant ChromatoProbe/GC-MS switching [21]. The ChromatoProbe (Agilent name "Thermal Separation Probe") is based on the conversion of a GC injector into an MS probe that accepts samples in micro-vials when the injector is connected to the ion source via a short 1 m micro bore (0.1 mm I.D.) transfer-line. The ChromatoProbe also uniquely enables the injection of very "dirty" samples without any sample preparation [21] when it serves as a thermal desorption unit behind a standard analytical column. The ChromatoProbe further excels in having relatively large micro vials with 2.4 mm I.D. that are easy to work with unlike those of MS probes of standard EI with 0.6-0.8 mm I.D. The use of the ChromatoProbe with Cold EI and its high flow rate with short transfer-line capillary enables significantly extended range of compounds amenable for analysis and combined with the fully inert Cold EI ion source and the provision of enhanced molecular ions the ChromatoProbe operation as an MS probe excels and is more effective and informative with Cold EI than a standard MS Probe is with a standard EI ion source. Similarly, intra injector thermal desorption is more effective and can be performed at lower injector temperatures when operated with the higher column flow rates that are possible with Cold EI.
43. **Tal Aviv Molecule Identifier Software (TAMI).** The TAMI software [37] utilizes several algorithms which improve the quadrupole mass accuracy and invert the measured mass, together with the molecular ion's isotopomeric pattern, into elemental formulae. TAMI automatically supports or rejects library identification results, and in a case of rejection can quickly provide the compound's elemental formula. For every independent analysis, TAMI provides a table of elemental formulae with declining order of matching to the experimental data, and presents additional statistical information. TAMI also includes a unique smart-analysis assistant that provides help in more complex cases, making sure the analysis conditions are optimal. TAMI Improves the mass accuracy of quadrupole mass analyzers to <100 ppm (typically 40 ppm), and in combination with isotope abundance analysis upgrades these unit resolution mass spectrometers to be similar in sample identification capability to costly accurate mass GC-TOF-MS with 1 ppm mass accuracy. However, while TAMI works well in standard GC-MSs it particularly excels with Cold EI as the provision of elemental formula requires having abundant molecular ions as obtained with Cold EI. In addition, the use of TAMI benefits from the extended range of compounds amenable for analysis in Cold EI. Thus, a quadrupole based GC-MS system coupled with Cold EI is more effective in the provision of elemental formulae than any expensive GC-MS with high resolution TOF and standard EI.
44. **Pulsed Flow Modulation GCxGC-MS.** An effective type of GCxGC modulation method named pulsed flow modulation (PFM) was developed in our lab for its combination with GC-MS [38, 39]. It is a simple and low cost type of GCxGC modulation method and device that does not require any cryogenic gas or liquid. However, it requires compatibility with second GCxGC column flow rates of ~20 ml/min which is not a problem with FID, FPD or other types of GC detectors but is incompatible with standard GC-MS flow acceptance that as a result require significant

flow splitting. However, Cold EI is seamlessly compatible with the pulsed flow modulation GCxGC flow rate requirements, providing the ultimate in both sensitivity and sample information. PFM GCxGC-MS with Cold EI further provides full elimination of second column capacity saturation and full elimination of ion source related peak tailing which is of importance in GCxGC-MS. Recent PFM-GCxGC-MS with Cold EI applications discuss the new concept of GCxGCxMS [40] and universal pesticide analysis [41].

45. **Low Thermal Mass Fast GC for ultra-fast GC-MS.** Currently, a few low thermal mass fast GC systems are available. The most widely used is of Agilent (previously RVM). Aviv Analytical provides its unique LTM Fast GC that enables full analysis cycle times of under one minute (50-350-50°C) with temperature programming rates of up to 2000°C/min and cooling back time of under 20 seconds [24]. This LTM Fast GC is fully compatible with the use of any fused silica short column and high column flow rate as described and demonstrated in [24]. However, one other feature that needs to be improved in fast GC-MS is the ability to perform fast splittless injections which require high column flow rates. Furthermore, fast GC-MS analysis of compounds in complex mixture requires the ability to periodically trim the column due to matrix contamination buildup at the front segment of the column near the liner, and to be able to replace the column at low cost. All the above mentioned improvements are uniquely met with the combination of our LTM Fast GC and GC-MS with Cold EI [24] and the feature of enhanced molecular ion of Cold EI improves the selectivity to offset the inevitably lost GC separation capability with short columns.

46. **Open Probe Fast GC-MS – Real time analysis with separation and library identification.** The ultimate goal in fast analysis is to combine fast separation with fast or no sample preparation in order to approach real time analysis with separation. Recently, a few types of real time analysis techniques such as DART and DESI gained popularity, but these methods suffer from several deficiencies and do not provide separation and/or library based easy and trustworthy identification. We have combined our unique low thermal mass fast GC-MS [24] with Cold EI together with a novel Open Probe inlet for achieving fast sampling without sample preparation [25]. The Open Probe is a probe-oven that is mounted onto the fast GC and which is open to room air with helium purge flow protection to eliminate air leakage to the fast GC columns and MS ion source. Thus, sample handling and introduction is as simple and fast as touching the sample (with a swab or melting point glass tube) and pushing it into the open probe. The Open Probe Fast GC-MS provides real time analysis [26, 27] and in comparison with DART and other types of real time analysis it uniquely provides the following features and benefits:

    1. Fast chromatography separation for improved mixtures analysis.
    2. Library based sample identification is enabled for the provision of the sample name and structure at the isomer level.
    3. TAMI software helps in the confirmation of library identification and in the provision of elemental formula for compounds that are not in the library and that exhibit molecular ion as in Cold EI.
    4. Cold EI uniquely provides uniform compound independent response for improved quantitation. Furthermore, quantitation by Cold EI does not suffer as ESI or APCI from any ion suppression effects.
    5. Extended range of thermally labile and low volatility compounds are amenable for analysis in Open Probe Fast GC-MS with Cold EI.
    6. Swabs can be used to bring samples from remote surfaces combined with full thermal desorption.

7. An in-vacuum ion source is used hence the instrument cost less than that of a high resolution MS of other real time analysis instruments. Furthermore, the broad install base of Agilent 5975/7 GC-MS can serve for the accommodation of Open Probe Fast GC-MS yet it excels with Cold EI.
8. The same system can be operated with a second injector as GC-MS.
9. No solvent is used unlike with DESI and the helium gas consumption is about 50 times lower than in DART.

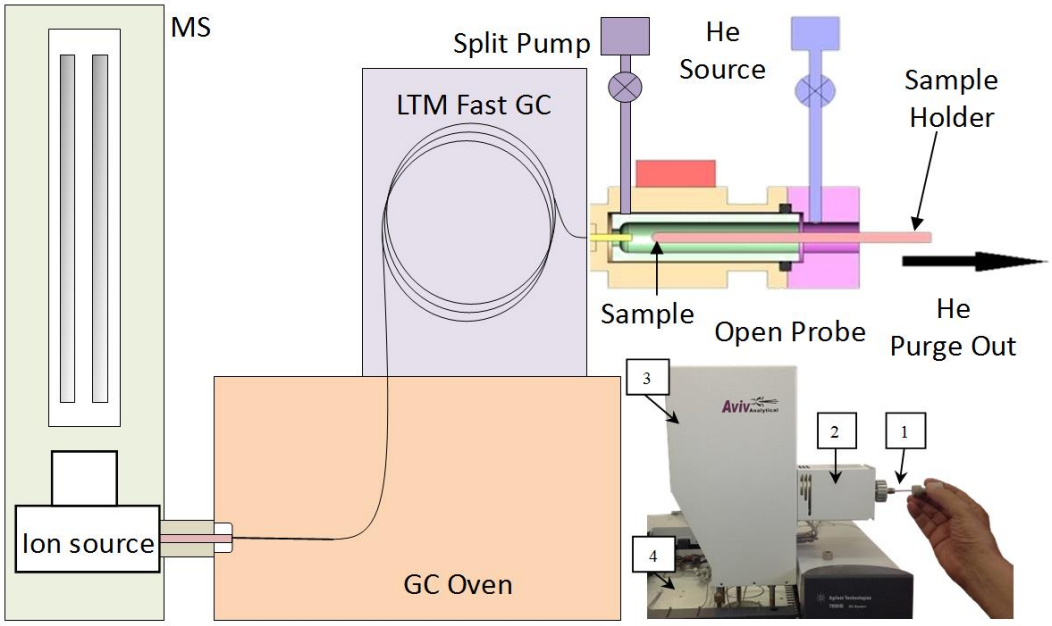

Figure 12. A schematic diagram of the Open Probe Fast GC-MS and a photo (bottom right side) demonstrating sample introduction using a melting point vial. The various components of the systems are indicated with names.

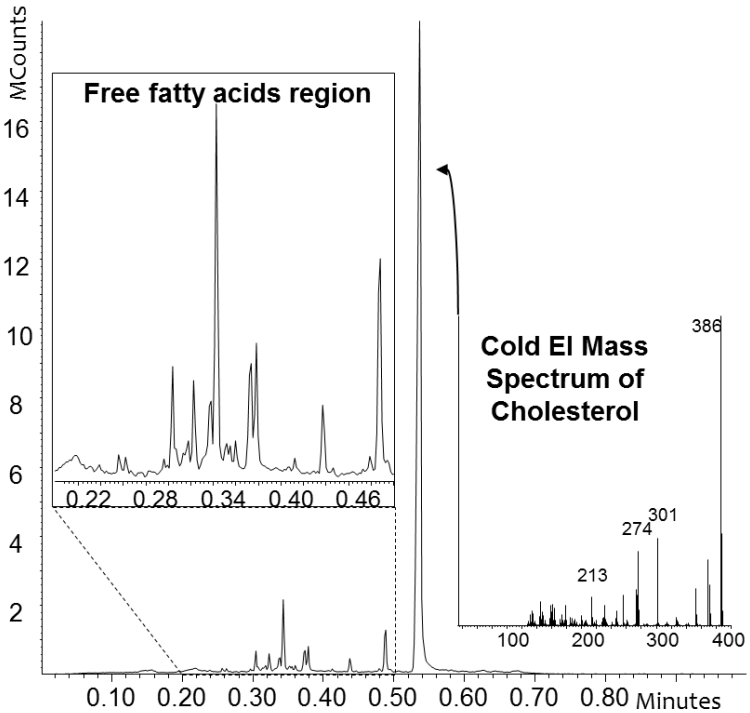

Figure 13. The analysis of blood for its cholesterol and free fatty acids content by Open Probe Fast GC-MS with Cold EI. A volunteer used a diabetes lancet to draw blood from his finger and a melting point capillary vial to collect a small drop (about 0.5 µL) and inserted it into the Open Probe for its thermal desorption and fast GC-MS with Cold EI analysis. Note the sub one minute analysis time.

In short, Open Probe Fast GC-MS [26, 27] reaches the ideal goal of achieving real time analysis with separation and library based identification, and with Cold EI it brings real time analysis to new frontiers. For a video presentation of Open Probe Fast GC-MS you can watch https://www.youtube.com/watch?v=73kvzXP7JdE Recently, Agilent introduced the Open Probe Fast GC-MS (without Cold EI) under the name QuickProbe Fast GC-MS

47. **Electron Ionization LC-MS in GC-MS.** LC-MS can significantly benefit from having electron ionization as it provides automated library identification and extensive fragment ions information for improved sample identification. In addition, EI does not suffer from ion suppression effects that plague ESI or APCI. Cold EI uniquely exhibits uniform and compound independent ionization yield (in contrast to ESI) for improved quantitation [36]. Thus, bringing back EI to LC-MS is highly valuable if a reliable and robust EI interface can be developed. Furthermore, yet another highly desirable goal is to have both GC-MS and EI-LC-MS in one system with easy method based switching between these two modes of operation. We developed a novel EI-LC-MS approach, based on interfacing LC and MS with supersonic molecular beams (SMB) and sample ionization with electrons as vibrationally cold compounds in the SMB (Cold EI) [42, 43]. The LC effluents are pneumatically sprayed inside a heated GC liner, followed by fast sample thermal vaporization at ~1-1.5 atm. The vaporization chamber is connected to the supersonic nozzle via a flow restrictor fused silica capillary. The original EI ion source was replaced by a fly-through Cold EI ion source and the ions were subsequently deflected into the mass analyzer and then detected. We also mounted the EI-LC-MS interface onto a GC detector slot in a GC-MS with Cold EI system thus have a dual system of GC-MS and LC-MS with Cold EI with method based mode changing. Consequently, in a GC-MS with Cold EI one injector slot can serve for GC-MS analysis and a modified detector slot for EI-LC-MS.
48. **Backflush.** Backflush is a known effective technique to maintain clean GC columns when used in the analysis of complex matrices. In backflush, at the end of the analysis, when the GC oven is at its highest temperature, or after the elution of the last to elute sample compound of interest, the flow in the column is inverted (reversed) via the provision of high pressure at the end (or middle) of the column and low pressure at the injector. As a result, heavy compounds with low volatility that coat the early portion of the column migrate back the short distance to the injector and this way are removed from the column. However, we found that some of the currently used backflush devices are active and in-time develop major peak tailing. Several backflush devices are based on a metal structure that is deactivated by Silcosteel (Restek) or another form of thin fused silica film. This film can deteriorate after several heating and cooling cycles due to large differences in the thermal expansion coefficients of fused silica and stainless steel. Thus, while in sales demonstrations backflush is very effective, after short usage some devices become faulty and induce major peak tailing for polar compounds. We developed a unique ultimate inert backflush device via the use of a simple 1/16" Swagelok T union that includes in its straight path a glass tube with 1.2 mm O.D. and 0.7 mm ID. The column and transfer line ends are brought inside this glass tube to a distance of ~1-2 mm while the third (middle) input serves to bring about 1-2 ml/min make-up helium gas from an EFC. This way, the added gas flow rate dynamically focuses the output of the column into the transfer-line and the sample compounds do not adsorb on any surface except the column and transfer-line hence tailing is eliminated. This novel concept of gas dynamic flow focusing into a column was described and demonstrated in our papers on pulsed flow modulation GCxGC [38, 39]. While our backflush device is inert it requires the addition of some helium flow rate towards the

ion source, which is not an issue with GC-MS with Cold EI but could be a downside in standard GC-MS. Backflush further excels with Cold EI in view of the ability to use high forward flow rate hence similarly high backflush flow rate.

49. **Thermal desorption and purge and trap.** While these known devices are effective their compatibility with GC-MS can be improved via the increase of splitless column flow rate acceptance as provided with GC-MS with Cold EI plus all the other benefits of Cold EI.

50. **Chemical Ionization (CI).** While CI is often needed in GC-MS analysis in view of the weakness or absence of molecular ions in standard EI, Cold EI is far superior to CI in many ways including: a) It is far more sensitive than CI; b) Changing mode from Cold EI to standard EI does not require any venting and ion source replacement and/or any addition of CI gas although usually such mode changing is rarely or never needed; c) Cold EI and classical EI-SMB use the same ion source thus Cold EI does not add price to classical EI for systems with Cold EI; d) Cold EI is the only soft ionization method that is compatible with NIST library identification; e) Cold EI provides uniform response unlike CI which is weak for hydrocarbons and other compounds; f) Cold EI extends the range of compounds amenable for GC-MS analysis while CI reduces it via its close structure. In short, Cold EI is far superior to CI and with it there is no need for CI.

51. Improved GC-MS-MS performance. MS-MS is a powerful GC-MS enhancement technology which helps particularly in the reduction of matrix interference in the analysis of target sample compounds in complex matrices. The major use of GC-MS-MS is in pesticide analysis in agricultural products and drug analysis in biological fluids. Cold EI improves GC-MS-MS performance in a few important aspects including: A) **Improved selectivity**. Cold EI enhances the abundance of the molecular ion (and its isotopomers) which is the most selective ion in the mass spectrum. Furthermore, when the molecular ion serves in MS-MS as the parent ion the daughter ion mass is typically higher than when a fragment is used as the parent ion. Consequently, the MS-MS selectivity is significantly improved by an estimated two orders of magnitude via the use of molecular ion instead of a fragment ion as the MS-MS parent ion and the proof of that fact is that LC-MS-MS is known to be more selective hence sensitive than GC-MS-MS. B) **Improved instrument sensitivity**. While MS-MS on the molecular ion further reduces matrix interference it also serves to increase the daughter ions signal hence the instrument sensitivity. This improved MS-MS sensitivity emerges in two ways of: 1) Molecular ions as parent MS-MS ions require lower CID voltage and they dissociate in the CID process into lower number of fragment ions which are better retained by the RF only Q2. The molecular ion is easier to dissociate than a stable fragment ion that was formed in the EI process since abundant fragments are abundant as they are typically stable fragments and thus are harder to break. The higher typical CID voltage used with fragment ions creates more energetic lower mass daughter ions that are harder to retain in Q2. 2) The increased selectivity of MS-MS on the molecular ion can be translated into up to a factor of four higher signal via the use of lower Q1 and Q3 resolution. C) **Extended range of compounds amenable for GC-MS-MS analysis.** GC-MS-MS is mostly used with groups of target compounds such as pesticides and drugs, which include significant portion of thermally labile compounds. As a result, GC-MS-MS suffers from growing competition with LC-MS-MS on those types of analyses. Cold EI enables the analysis of much greater range of those pesticides and drugs and can even serve for the analysis of pesticides that are difficult to analysis by both GC-MS-MS and LC-MS-MS such as captan, captafol and folpet. Furthermore, GC-MS-MS with Cold EI can uniquely serve for the confirmation of LC-MS-MS labile samples. D) **Faster GC-**

MS-MS analysis. Since the selectivity against matrix interference is improved with Cold EI, one may wish to translate the added selectivity into faster analysis. Cold EI can serve to achieve much faster GC-MS-MS analysis all the way to less than one minute ultra-fast GC-MS-MS full analysis cycle time as demonstrated in [24]. In addition, many of the GC-MS improvements listed in this paper are applicable for GC-MS-MS.

52. **Photoionization of cold molecules in SMB (Cold PI).** We recently developed a new type of photoionization ion source of cold molecules in supersonic molecular beams (named Cold PI). The base system was a GC-MS with supersonic molecular beams and its fly-through EI Cold EI ion source that was modified to include a continuously operated deuterium VUV photoionization lamp between the nozzle and skimmer. We found that Cold PI is far softer then photoionization of thermal compounds and as soft as field ionization yet it is stable and works well in GC-MS with Cold EI and with quadruple MS. Practically all hydrocarbons gave only molecular ions while alcohols gave some molecular ions plus major fragment ions particularly with a loss of water (similarly to field ionization). We tested Cold PI in the GC-MS analysis of Diesel fuels and analyzed the time averaged data for group type information. We also found that we can analyze Diesel fuels by fast under 20 s flow injection analysis in which the generated averaged mass spectrum of molecular ions only could similarly serve for fuels characterization. Accordingly, Cold PI is another enhancement technology of Cold EI. A manuscript on Cold PI is under preparation [44].

G. Improved GC-MS Maintenance, Flexibility, Ease of Use and Price

While not often discussed, maintenance, flexibility, ease of use and price are all important GC-MS parameters that can be improved and this section describes several areas of their possible improvements.

53. **Unlimited selection of column parameters.** In LC-MS unlike in GC-MS users can select broad range of columns with various lengths, diameters, solvent types and solvent flow rates while GC-MS is practically restricted to 30 m columns with 1 ml/min helium flow rate. In GC-MS with Cold EI, any column can be used without restrictions on its diameter, length and flow rate. This feature allows optimal trade-off of GC resolution, speed, sensitivity and range of compounds amenable for analysis and it significantly simplifies analysis method development.
54. **Number of columns that can be simultaneously connected.** In GC-MS with Cold EI two columns can be simultaneously connected with the nozzle transfer line from two different injectors and the added flow rate has no adverse effect on the system performance. Even three columns can be simultaneously connected in GC-MS with Cold EI with an additional third injector. This feature improves the GC-MS flexibility in a few ways such as enabling fast screening with a short column followed by confirmation with a longer column. Similarly, it enables the use of one injector with the ChromatoProbe either as an MS probe or behind a separation column for intra injector thermal desorption of solids or sludge samples while a second injector can serve for standard syringe based liquid injections. In addition, one injector and column can serve for GC-MS analysis while the second injector can be modified and serve for EI-LC-MS or flow injection analysis as described in 46 above. In short, the ability of having two simultaneously connected columns with the nozzle and Cold EI ion source is highly desirable as it improves the system's flexibility.
55. **Columns replacement and injector service without breaking vacuum.** One further aspect in which GC-MS can be improved is to make its service easier via enabling the replacement of its GC column and injector liner or septum without venting the MS

vacuum chamber and/or full injector and transfer line cooling. For GC-MS with standard EI a few "no-vent" devices were developed which require the addition of another EFC and added cost. In GC-MS with Cold EI the column output is designed to be able to tolerate atmospheric pressure and the make-up gas EFC is already available. Thus, during column replacement the nozzle flow rate is increased to form nozzle pressure of about 1100 mBar while the injector flow rate is off (1 Bar pressure). As a result, the column flow is reversed and thus liner or septa can be replaced while the column is protected from air penetration particularly at its heated transfer-line zone. Similarly, when the transfer-line is open for column replacement helium flows out and purge protects the transfer-line from the penetration of air although small amount of air flow is actually harmless to the fly-through ion source when its filament is off. We note that some GC-MS users service the injector without cooling the transfer line and ion source. This is a mistake as air at the hot transfer line damages the portion of the column in it which becomes active and increases its bleed plus the air does not benefit the hot standard EI ion source.

56. **Column flow programming and no column flow rate effects on ion source response.** In standard EI the ion source is designed to maximize the sample ionization yield at about 1 or 1.2 ml/min helium flow rate. Above this flow rate the ion source response begins to decline, so that a new tune is required, and above a few ml/min the ion source response is sharply reduced due to extended intra-ion-source space-charge effects. In contrast, Cold EI has no ion source flow rate effects. In Cold EI the nozzle back pressure is stabilized at values of about 1 Bar (usually at 830 mBar) and as the column flow rate is modified, the added helium make up gas flow rate is automatically changed to maintain and stabilize the set nozzle back pressure. Thus, the supersonic molecular beam pressure and effective helium flow rate at the ion source is independent on the column flow rate and consequently the Cold EI ion source response is column flow rate independent. This feature opens new and unique opportunities with column flow programming to improve the range of compounds amenable for analysis, to keep the column clean from matrix compound deposits and to speed-up the analysis as demonstrated and described for Heroin analysis [19].
57. **Multiple ion sources operation modes and their fast changeover.** In most standard GC-MS (excluding ion traps) systems the replacement of standard EI ion source with that of CI is lengthy and requires venting and hardware change plus added price. In GC-MS with Cold EI the same fly-through ion source can serve in four modes of operation that are interchangeable via a method change which takes a few seconds or minutes, without any hardware change and without added cost. The fly-through ion source can be operated in the following four modes of Cold EI, Low Electron Energy "Soft" Cold EI, Classical EI-SMB and Cluster CI. However, it is preferable to be able to work with only one ion source, and the fly-through ion source in its Cold EI mode of operation is close to the ideal ion source that outperforms both standard EI and CI ion sources combined as elaborated throughout this paper.
58. **Temperature programmable transfer line.** GC-MS transfer-lines are currently provided by all vendors without temperature programming capability and thus are typically maintained at the upper GC oven temperature specified in the method such as 300°C. The Aviv Analytical 5975-SMB GC-MS with Cold EI is uniquely provided with transfer-line temperature programming capability to improve the GC-MS performance in several aspects: A) In the analysis of a mixture of several compounds some of which are relatively volatile thermally labile compound the transfer line is maintained at a relatively low initial temperature such as 180°C and only after the elution of the thermally labile compound(s) its temperature is increased to prevent peak broadening

for the late eluters. A typical example is the analysis of pesticides that include the relatively volatile thermally labile carbamate pesticides (aldicarb, methomyl etc.) as well as less volatile pesticides, and similarly explosives mixtures that include TATP; B) Lower initial transfer-line temperature results in lower PDMS transfer line bleed hence provide lower MS noise and increased sensitivity; C) Every syringe injection includes about 0.5-1 µL air in the empty portion of the syringe needle. Consequently, even if the column is cooled during the injection, the pure air that is inevitably injected interacts with the column at its transfer line section and induces PDMS bleeding noise that makes this portion of transfer-line column active with exposed silanol groups. The use of temperature programmable transfer-line significantly reduces this problem.

59. **Transfer-line temperature uniformity.** While not specified by any vendor, transfer-lines inherently suffer from having non–uniform temperature along their axis which requires their extra heating to prevent peak broadening which can induce delicate sample decomposition. While the GC side is actively cooled by the GC oven, the ion source side is not fully heated by the transfer-line heater due to limited heat transfer that depends on the design. In fact, since the ion source temperature is insulated from the transfer-line temperature a local cold spot between them is inevitably formed. The transfer-line of the Aviv Analytical 5975-SMB GC-MS with Cold EI is designed with thick aluminum block heater with 28 mm diameter for effective heat transfer to both sides. In addition, once the sample elutes behind the nozzle it is mixed with 60 ml/min make-up gas, thereby eliminating peak broadening at the inevitably cooler nozzle even if its temperature is 30°C lower. Furthermore, unlike in standard GC-MS transfer-lines, in GC-MS with Cold EI the nozzle is more effectively thermally coupled with the transfer line heater, thereby reducing any cold spot between them. At the GC side, an aluminum jacket transfers the heat from the transfer line heater block up to the column entrance point to minimize any "dynamic cold spot" that can be formed during fast GC oven temperature programs.

60. **High temperature GC operation in GC-MS.** In GC-MS with standard EI the current industry standard for upper transfer-line and ion source temperatures is 350°C. This upper limit restricts the range of low volatility compounds that can be analyzed and in addition, as the ion source temperature is increased, the relative abundances of the molecular ions are exponentially reduced. In the 5977-SMB GC-MS with Cold EI the ion source is of a fly-through design hence its temperature (typically 400°C) is irrelevant for the analysis. The transfer-line temperature is limited to 350°C although a higher temperature version was produced. As described above, the use of short columns with increased column flow rates enable the analysis of low volatility sample compounds up to and beyond the mass limit of the 5975/7 MSD of 1050 amu. However, the penalty for using shorter columns with high column flow rates is having somewhat reduced chromatographic separating by about a factor of 2 peak capacity per 40°C lower elution temperature. As a result, it is beneficial to analyze complex mixtures of stable low volatility compounds using high temperature standard length columns (15-60 m) with GC oven temperatures up to 420°C. The 5975-SMB GC-MS with Cold EI enables such high temperature analysis with a backflush T union device that adds flow rate to the transfer line, and thus with the use of 1 ml/min column flow rate and 16 ml/min added make up gas at the transfer line, the GC oven can be heated up to 420°C without transfer-line induced peak broadening even if the transfer line is at 350°C. As a result, low volatility compounds can be analyzed with improved separation.

61. **Compatibility with hydrogen and/or nitrogen carrier gases.** In certain cases the helium supply could be interrupted and one might wish to consider working with hydrogen or nitrogen as the carrier gas. As described in our blog article [45] the use of

hydrogen with standard EI could lead to the chemical activation of the GC liner and ion source, while the use of nitrogen significantly reduces the ion source ionization yield due to significantly (x7) increased ion source space charge. Cold EI can uniquely operate with nitrogen as the column carrier gas and hydrogen as the make-up gas with minimal loss in sensitivity as described in our blog post on this topic [46].

62. **Ion source robustness (and maintenance).** Ion source robustness is an important feature of any ion source. In standard EI the ion source requires periodic cleaning with an abrasive material to remove polymerized insulating material from its metal surfaces that can be charged by the ionizing electrons and distort the intra-ion-source electrical fields. Alternatively, one can add the Agilent Jet-Clean which adds cost and complexity. The Cold EI fly-through ion source is highly robust and requires very little maintenance since ~75% of the sample compounds are eliminated by the entrance skimmer and from the rest 90% fly through the ion source thus only 2% of the sample compounds scatter from the hot (400ºC) and large ion source surface area. In addition, the quadrupole also remains clean as the ion source is separated from the quadrupole mass analyzer by a 90º ion mirror. Our applications system 5975-SMB GC-MS with Cold EI works with its eight years old Cold EI ion source that was never serviced and still maintain its full performance the same as a new one. We note that most standard EI ion sources slowly reduce their performance and require maintenance, which is usually delayed. Moreover, after the ion source has been serviced its performance will never be like that of a new source due to its poorer internal surface quality. Cold EI on the other hand keeps its peak performance for years without service.
63. **Ion source temperature-independent mass spectra**. Obviously users want their GC-MS to provide reproducible mass spectra regardless of the ion source conditions and temperatures. However, the ion source temperature strongly affects the obtained standard EI mass spectra. For example, as demonstrated and explained in our post on peak tailing [20], the relative abundance of the molecular ion is exponentially reduced with the ion source temperature. In Cold EI the ion source temperature is irrelevant to the Cold EI mass spectra which are reproducibly obtained. In addition, the Cold EI fly-through ion source is self-cleaned and provides Cold EI MS with little or no effect of extended use.
64. **Bigger GC-MS system pump.** In recent years there was/is a trend of increasing the size of the GC-MS vacuum system pump and older systems with 70 L/s turbo molecular pumps were replaced by new GC-MS systems that are offered only with bigger 250 L/s pumps or split turbo molecular pumps for having differential pumping. In fact, currently all the major GC-MS vendors sell their systems either with big (performance) turbo molecular pump or with split (differentially pumped) turbo molecular pump. Clearly, with a bigger vacuum pump, pump down time is faster, vacuum background noise is lower and the maximum allowed input flow rate is higher. Thus, the competition on increased OFN specification leads to increased vacuum pump pumping speed despite the added cost. GC-MS with Cold EI includes an additional differentially pumped vacuum chamber with 250 L/s turbo molecular pump, allowing a record high column flow rate (up to 100 ml/min) and fully eliminates vacuum background, thus achieving the most from the addition of a differential pumping stage. This trend of using "big" or dual stage pumps reduces the added cost gap between standard GC-MS and GC-MS with Cold EI.
65. **Easier and more flexible method development.** For optimal GC-MS operation its method of operation should be tailored to the analysis task. For example, in service GC-MS samples significantly vary in terms of sample types, volatility, thermal stability, and the need for side products identification. As a result, an improved GC-MS system

should enable flexible and easy method development, particularly in terms of flow programming and the use of short columns as provided by GC-MS with Cold EI. As an example, we typically use 15 m 0.32 mm I.D. column with 0.1 µ DB1-HT film that can accept flow rates in the range of 1 up to 32 ml/min plus provide much faster analysis. While its separation is lower by a factor of 1.5 from standard 30 m columns Cold EI provides superior separation for large compounds via the elimination of ion source peak tailing and far greater method flexibility.

66. **Demonstration of benefits in challenging applications and new analysis methods.** Every GC-MS vendor praises its system with all the buzzwords and superlatives. They also provide application notes, but these notes mostly demonstrate their system use in standard applications and thus are mostly a "me too" type of statement. However, GC-MS systems should be further evaluated via the availability of application notes and demonstrations of the claimed benefits in the analysis of ***challenging*** as well as real life applications. Currently 56 unique and challenging applications are published in the Advanced GC-MS Blog Journal [47]. Furthermore, with Cold EI multiple benefits are sometimes combined into the development of unique new analysis methods such as isomer distribution analysis [8], universal pesticide analysis method [41], the determination of chemical reaction yields [36] and a few other methods as described in several blog posts. Finally, the ultimate system test is in its applicability to user's specific applications and goals based on user samples that may serve as the ultimate system performance test and demonstration.
67. **Price.** This is the bottom line for many users. The price of GC-MS with standard EI ion source is moderate. The price of GC-MS with Cold EI ion source can be similar to that of standard EI if a fully integrated ion source will be available as can be achieved by a company that will produce a full GC-MS with Cold EI system. The Aviv Analytical 5975/7-SMB GC-MS with Cold EI serves as an add-on system and thus increases the price of single quad GC-MS but this high price emerges not from the technology itself but rather from the fact that many of its components are doubled (vacuum chamber, turbo pump, ion source, transfer line etc). A few Cold EI price consideration include: A) While the Cold EI requires one split turbo molecular pump it can be operated with lower size and pumping speed pump such as with 150 L/s or even 80 L/s as its OFN specs are unaffected by the pump size; B) Cold EI requires the addition of one EFC/EPC (electronic flow/pressure control); C) Cold EI does not work with negative ions thus all the negative ion CI compatible power supplies can be price reduced for positive ions only operation; D) Cold EI is a robust ion source thus no service items such as airlock for its replacement without venting or "Jet Clean" are needed which lowers its cost of goods; E) Cold EI does not require an expensive MS probe for the analysis of thermally labile compounds and even if desirable for mass spectral studies it works well with the lower cost ChromatoProbe; F) Cold EI does not require CI or any other ion source. G) Cold EI does not require any no-vent device for enabling column replacement without venting. H) Cold EI in combination with the TAMI software enables the effective generation of elemental formulae and thus brings the value of expensive high resolution MS in a much lower cost GC-MS system and for greater range of compounds. Thus, if adopted by a major vendor the cost of goods of GC-MS with Cold EI can be similar to those of GC-MS systems with standard EI.

H. Improved Utilization of Mass Analyzer Specifications

The mass analyzer specifications clearly belong to "what can be improved in GC-MS". Initially, even though these mass analyzer specifications are not affected by the interface and ion source performance, they are coupled and the improvement of an interface's

characteristics can increase the benefits provided by the mass analyzer. Thus, a close examination of these aspects reveals that in fact Cold EI enhances the benefits from and utilization of all the mass analyzer specifications.

68. **Improved benefit from extended mass range.** The mass range is a parameter that is specified by all GC-MS vendors. Typically it is from low mass up to and over 1050 amu in quadrupole based GC-MS. While such mass range is sufficient for standard GC-MS operation since very few compounds with molecular mass of over 1050 amu can elute from standard GC-MS column, GC-MS with Cold EI can serve for the analysis of much bigger compounds (about doubled mass range) and thus can benefit from higher mass range specification while uniquely utilizing the full mass range of currently used mass analyzers. We note that such large compounds with mass over 1050 amu not only can elute but they also provide useful molecular ions in Cold EI.
69. **Doubled mass spectral range via availability of doubly charged molecular ions.** Doubly charged molecular ions are initially produced at about 20-30% of the ions but while they are observed in atoms and large polycyclic aromatic hydrocarbons they fully dissociate into two singly charged fragment ions in the vast majority of molecular ions in standard EI. On the other hand in Cold EI most of the large compounds including large aliphatic compounds with >C40 exhibit abundant doubly charged molecular ion. Such M^{+2} ions double the effective mass spectral range of the mass analyzer and enables Cold EI analysis of compounds up to and over 2000 amu. In addition they may add some isomer structural information

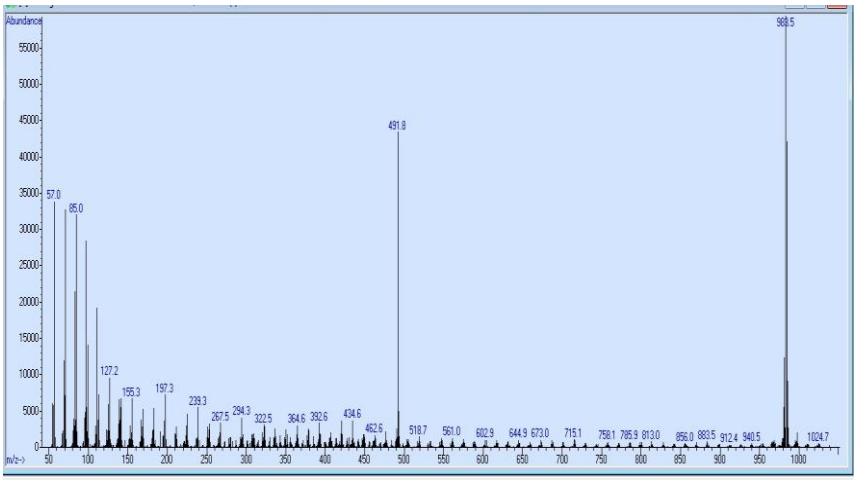

Figure 14. Single quad based GC-MS with Cold EI provides enhanced doubly charged molecular ions that double the effective mass spectral range. The figure shows the Cold EI mass spectrum of n-$C_{70}H_{142}$ (M = 983.1) with the doubly charged molecular ion at m/z = 491.6

70. **Improved benefit from higher mass resolution.** Higher mass resolution serves mostly for the reduction of matrix interference as well as for the provision of elemental formula. However, in order to benefit from the reduction of matrix interference the sample compound must first elute and as described above the Cold-EI interface enables the analysis of significantly greater range of compounds. Furthermore, Cold EI also lowers matrix interference via the enhancement of the molecular ions as described in [30] and in our blog post on selectivity enhancement [31]. As a result, the effect of high resolution is amplified with Cold EI and the combination of high resolution and enhanced molecular ions can provide the ultimate selectivity and matrix interference rejection or alternatively be translated to much shorter analysis time.
71. **Improved benefit from higher mass accuracy.** High mass accuracy serves for the elucidation of elemental formulas. However, for such service, the sample compound must first elute and than it must exhibit a trustworthy molecular ion. Cold EI excels in

comparison with standard EI in both of these aspects thus amplifies the benefits of high mass accuracy. In addition, our TAMI software [37] can combine the high mass accuracy with isotope abundance analysis for obtaining further improved provision of elemental formula.

72. **Improved benefit from faster scan speed.** Scan speed is a typical specification highlighted by those vendors who sell GC-MS with time of flight MS. However, the reality is that for fast GC-MS there is no need for scan speeds that are faster than that which is provided by current modern quadrupole MS, which is 20,000 amu/s (40 Hz for mass range of 50-550 amu). Only for GCxGC-MS with thermal modulation and/or with ultra-fast GC-MS with sub one minute analysis it is desirable to have higher scan speed. However, fast scan speed alone is insufficient for GCxGC-MS since for semi-volatile, low volatility and polar compounds ion source related peek tailing broadens the GC peaks and hampers the GCxGC resolution. As a result, the ion source temperature must be increased to reduce this ion source peak tailing in order to benefit from the fast scan speed and narrow peaks. However, one must remember that an order of magnitude narrower peaks (as claimed for GCxGC-MS by the TOF companies) requires 70°C higher ion source temperature than what is needed for standard GC-MS and this 70°C higher ion source temperature exponentially reduces the molecular ions and mass spectral information content plus induce ion source degradation for many labile compounds. Cold EI eliminates these problems, provides sub millisecond ion source response time regardless of the sample compounds' volatility or polarity (due to its fly-through operation) and also provides enhanced molecular ions without ion source temperature issues. Thus, since GCxGC-MS is all about information, faster scan speed shines brighter with Cold EI as their combination uniquely enables the analysis of very narrow GCxGC peaks of semi-volatile compounds.

Discussion

This article elaborates on the subject of "what can be improved in GC-MS" and describes seventy two areas of improvements and how they can be obtained via the use of the GC-MS with Cold EI with its supersonic molecular beams interface, fly-through ion source and other supporting enhancement technologies. GC-MS with Cold EI is characterized by the following major 20 improvements and benefits:

1. Significantly extended range of compounds amenable for analysis.
2. Enhanced molecular ions and high-mass structurally-informative fragment ions.
3. The only soft ionization method that is fully compatible with NIST library identification.
4. Uniform response for improved quantitation and provision of chemical reaction yields.
5. Same ion source provides Classical EI, Cold EI and low eV Soft Cold EI mass spectra with method based mode changing.
6. Best identification and provision of elemental formula with the TAMI software by providing enhanced molecular ions for greater range of compounds.
7. Much faster analysis is enabled.
8. Column flow programming is provided without affecting sensitivity.
9. Improved sensitivity particularly for difficult to analyze compounds.
10. Vacuum background elimination thus having the lowest vacuum background noise.

11. Highest (by far) ratio of TIC peaks to column bleed (better S/N and ID).
12. Inherently inert ion source even for low pg range polar compounds.
13. No peak tailing for better signal as well as improved chromatography and separation.
14. Isomer distribution analysis is uniquely enabled.
15. Doubly charged molecular ions are provided for doubled mass spectral range.
16. Fastest (by far) ion source response time.
17. Improved selectivity and thus reduced matrix interference on the molecular ions.
18. Best compatibility with pulsed flow modulation GCxGC-MS and a range of additional GC-MS enhancement technologies.
19. Robust ion source that rarely needs cleaning.
20. Better linearity and reproducibility at low levels.

Naturally, some improvements are more important than others, and we consider the extension of the range of compounds amenable for analysis as the most important advantage of GC-MS with Cold EI since it bridges the gap between GC-MS and LC-MS and opens-up new areas of analysis and applications. The feature of enhanced molecular ion combined with NIST library identification is considered by us as the second most important Cold EI benefit while the Cold EI response uniformity is ranked #3. As a result, GC-MS with Cold EI can induce total GC-MS applicability and market growth.

Quoting Aristotle "The whole is greater than the sum of its parts", the combination of so many improvements (>60) creates a new and qualitatively superior technology that actually improves every type of analysis. While GC-MS with Cold EI enables new type of analyses and significantly improves challenging analyses it does not impede on any simple method of analysis (compared with standard EI) and its added cost could be negligible or none in a fully integrated GC-MS with Cold EI. Consequently, GC-MS with Cold EI is destined to lead the way for the future of GC-MS.

As a good closure of this article we quote Freeman Dyson from his book "Imagined Worlds":

"New directions in science are launched by new tools much more often than by new concepts. The effect of a concept-driven revolution is to explain old things in new ways. The effect of a tool-driven revolution is to discover new things that have to be explained."

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