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NIST 11

What's New and What Value Does it Offer? Part 1



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O. David
Sparkman

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In 1971, shortly after the acquisition of its initial six GC/MS instruments, the newly formed United States Government's Environmental Protection Agency (EPA) awarded a development contract to Battelle-Memorial Institute in Columbus, Ohio. This contract was to create a system that automatically transmitted data over voice-grade telephone lines from minicomputers connected to GC/MS instruments in field laboratories to a program running on a large-scale remote time-sharing computer, where a search was performed against m/z value and intensity pairs stored as fields in each individual spectrum, which constituted records in the database. The mass spectral search software was based on the Hites and Biemann search developed at MIT[1] where the m/z value and the intensity of the two most intense peaks in a window of every 14 m/z units (starting at m/z 5) was used for the comparison. The names of compounds whose spectra were similar to the spectrum of the unknown were automatically printed at the user's terminal. These names were printed in order of the similarity of their spectra to the spectrum of the unknown. A numerical similarity scale of 0 to 1 was established and included with the printout. The goal was to be able to provide a tentative identification without the need of a highly trained mass spectrometrist. The EPA Database was made up of spectra from

the Mass Spectrometry Data Centre (MSDC), an agency of the British government located at Aldermaston, England. This collection of spectra included a number of smaller collections: the American Petroleum Institute's file of mass spectra, the Dow Chemical Company's collection, an American Society for Testing and Materials (ASTM) collection, and several other smaller sets of spectra. This original collection had ~10,600 spectra including an unknown number of replicate spectra, some of which may have been duplicates. The EPA added 600 spectra of identified pollutants. A significant limitation of the EPA system, which was also a limitation of the first database search programs used on the minicomputer data systems provided by mass spectrometer manufacturers, was that a spectrum of the compound needed to be submitted for a spectrum (and any associate information; i.e., elemental composition, nominal mass, Chemical Abstracts Registry number (CASrn), structure, etc.) of that compound in the database to be returned.

At about the same time, the National Heart and Lung Institute (NHLI) of the National Institutes of Health (NIH), another agency of the U.S. Government, was implementing a similar mass spectral identification system using a somewhat updated MSDS Database (a.k.a. the Aldermaston collection) where m/z -intensity pairs of a sample

Figure 1

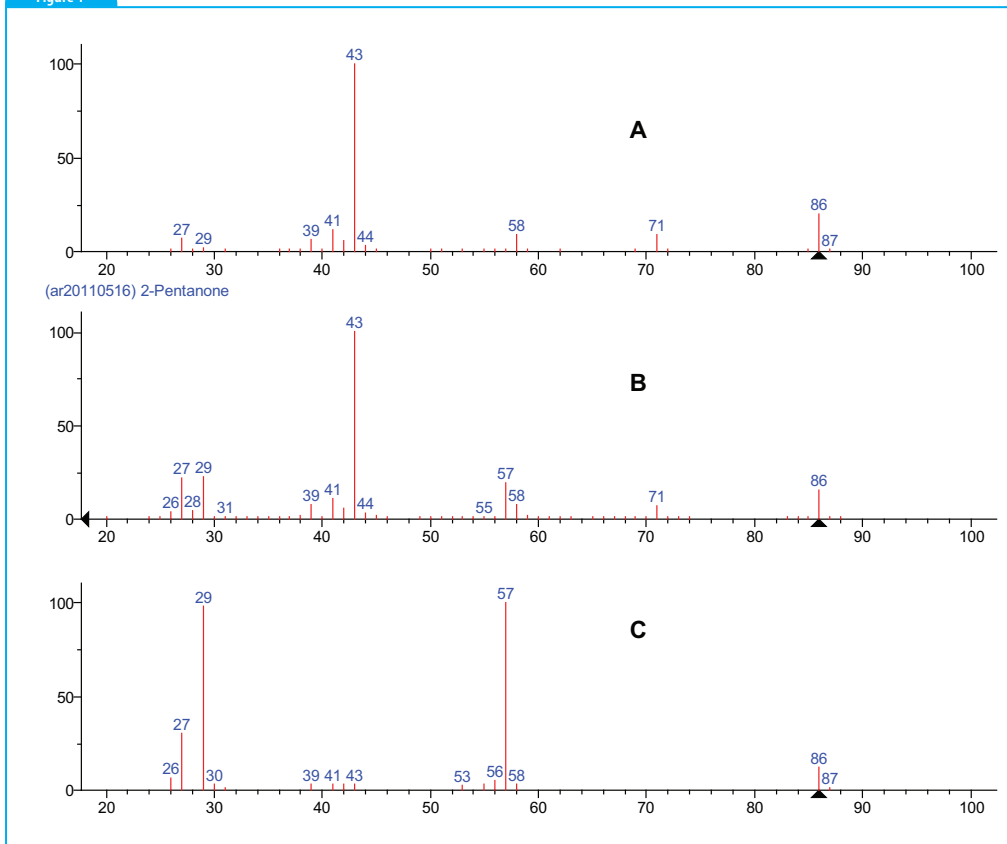


Figure 1: (A) Spectrum of 2-pentanone where all peaks can be rationalized by the structure. (B) Spectrum of 2-pentanone in the NIST 08 replib Database showing peaks at m/z 29 and m/z 57 which cannot be rationalized by mechanistic interpretation. (C) Spectrum of 3-pentanone where all peaks can be rationalized by the structure. (B) appears to be a spectrum of a mixture of 2-pentanone and 3-pentanone.

spectrum were entered one at a time using a remote terminal and voice-grade telephone lines connected to the mainframe storing the database. It was not necessary that the terminal (which functioned as a keyboard and printer) be connected to the GC/MS systems. The number of spectra in the database having m/z -intensity pairs with an intensity within a specified range of the entered values, was reported back to the user. Another pair was entered and the number of spectra having both pairs would be reported. This process would be repeated until there were a reasonable number of spectra, and the user would then request a printout of the name of each compound. This search algorithm became known as the Any Peaks Search which is still a key component of the *NIST Mass Spectral (MS) Search Program*. The NIH systems had several major advantages over the



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EPA system. The database could not only be searched by the Any Peaks method, but could also be searched by the nominal mass of a compound, a complete or partial elemental composition of a molecular ion, losses from a molecular ion, MSDS classification codes, or any combinations of these. The system also allowed for complete spectra to be output at the user's terminal either in a text or graphic format. Being able to retrieve the database spectrum in a graphical format allowed a comparison between the spectrum of the unknown and the database spectrum. This was especially helpful when there was not a spectrum of the unknown in the database and it was necessary to make comparisons to identify structural moieties in the unknown. This system was considered to be directed towards the experienced mass spectrometrist due to the fact that judgments were necessary.

Circa 1975, these two systems were merged. The database, which was now maintained by MSDC, was put on a commercial time-sharing computer system supported by a well developed communications network accessible by local telephone calls from many cities in the U.S. and overseas. [2] This project not only involved the original parties but was expanded to include the U.S. Federal Drug Administration (FDA). The merged database consisted of an expanded version of the original MSDC file, a collection acquired from John Wiley & Sons (the Wiley Registry of Mass Spectra), and spectra collected by EPA all of which resulted in ~30K spectra. The goal was to use other donations and contracts administered by the three U.S. Government agencies to develop a collection of ~50K spectra. This merged system allowed for the EPA transmittal of complete spectra and also the NIH multiple-function searches all using voice-grade telephone lines. This was known as the MSSS (Mass Spectral Search System). At the same time, the EPA Database of ~12,500 spectra was made available by a yearly subscription (of a magnetic tape) from the U.S. National Bureau of Standards (NBS), which later had its name changed to the National Institute of Standards and Technology (NIST). There was only one spectrum per compound. The complete spectra (spectra having all the peaks that had been acquired) on that tape were condensed by the instrument manufacturers to the two most intense peaks in a 14 *m/z* unit windows and used with second-generation GC/MS data systems that had two disk drives instead of the original DECTape. The only way a database spectrum could be retrieved would be through a database search of a spectrum of that compound that had been generated with the GC-MS.

Evolution of NIST's Distribution of EI Mass Spectra

By 1978, the NIST/EPA/NIH Mass Spectral Database contained 25,556 spectra; a 1980 release had 34,363 spectra; by 1992 the Database had grown to 74,828 spectra, 12,593 of which were selected replicates. The NIST 11*

*The NIST/EPA/NIH Mass Spectral Database has been distributed under many different names. In 1998, NIST began using the format NISTYY, where YY were the last two digits of the distribution year. There has been a NIST 98, NIST 02, NIST 05, NIST 08, and now NIST 11. Agilent used a naming convention that contained NBS (the erstwhile acronym for NIST) and the number of spectra; i.e., \NSB128K.L.



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Database contains 243,893 EI spectra of 212,961 compounds. Prior 1992, the Database contained only one spectrum per compound. This spectrum was considered to be the best quality spectrum for that compound in the NIST Archive Database; however, due to variances in EI spectra produced by different types of instrument (magnetic sector double-focusing vs transmission quadrupole; quadrupole ion trap vs time-of-flight, etc.) and that often times averages of several acquired spectra were compared against the Database, it was decided to include replicate spectra from different sources (different instruments and different laboratories using the same make and model of an instrument) for important compounds that exhibited such differences, but that had been evaluated and were judged to be quality spectra. The NIST 11 Database of EI spectra contains 30,932 replicate spectra of 19,788 compounds. The NIST Database is divided into two files: one contains only a single spectrum, which is considered the "best" spectrum for each compound. This file is actually a folder with the name \mainlib. The other file (also actually a folder) contains all the replicate spectra and has the name \replib. A compound may have only one replicate spectrum or it may have several replicate spectra. Three spectra in the \mainlib each have seven replicate spectra. For example, cocaine has a \mainlib spectrum and five \replib spectra where as N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]-urea has only one replicate.

Although the EPA/NIH Database was made available to instrument manufacturers through NBS (later known as NIST), the stewardship remained with the EPA until 1988. At that time, EPA turned the stewardship over to NIST. Based on what is known as the Standard Reference Data Act of 1968 (Public Law 90 -396) passed by the U.S. Senate and House of Representatives (90th Congress H. R. 6279, July 11, 1968) and signed into Law by the President of the United States, the U.S. Secretary of Commerce (NIST is part of the Department of Commerce) is mandated to collect and publish standard reference data and sell such data to recover the cost of the collection and publication of these data. This law is the reason that NIST charges its distributors, who in turn, charge their customers, royalties for the use of these mass spectral data. All based on this law, NIST began a program to ensure the quality of these data and to facilitate its ease of use.

At the time that NIST assumed stewardship of the Database, it was decided that the Database needed to be evaluated to assure that the spectra were actually what was stated. Because of the limitation of mass spectral search systems on instrument manufacturers data systems being able to only display a database spectrum when a sample spectrum was searched against the database, NIST needed to developed a search program to aid its workers to view and evaluate the data. This program was developed along the lines of the features available in the MSSS that was accessible on a time-share basis; however, it could be used with a personal computer. The first version of the **NIST Mass Spectral (MS) Search Program**

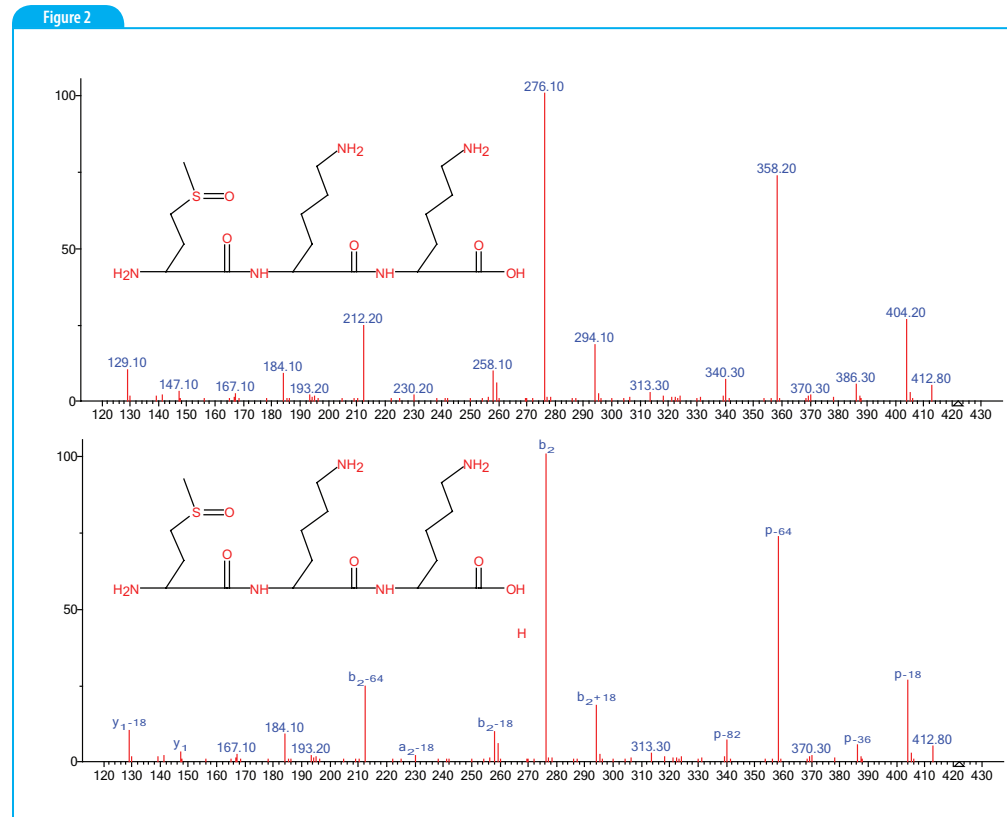


Figure 2: Two displays of the same mass spectrum from the NIST 11 MS/MS Database of a tripeptide. The top spectrum illustrates the normal peak labeling and the bottom spectrum illustrates the optional labeling of y and b ions.

was released in 1989. The **Program** was quickly embraced by the mass spectrometry community because it allowed for easy access to all the data in the Database. The **Program** continued to evolve as DOS-based software until 1995 when the first Windows-based version was developed. This Windows programs progressed through several version until 2001 when the current Windows version 2 was released. Enhancements and changes required to accommodate changes in the Windows computer operating system were made and released with each new version of the Database. Beginning with the release of the Database in 1998, NIST set as a goal to have a new release of the data every three years and consequently a new version of the **Program**. The next release was NIST 02 in 2002 with version 2.0a. Version 2.0g has been released with NIST 11.

In 1990, as NIST continued to acquire spectra from outside sources and measure spectra in its laboratories using a Hewlett Packard (now Agilent Technologies) GC Mass Selective Detector (MSD), and later a JEOL GCmate

(double-focusing instrument), an evaluation of the spectra in the Database began. This resulted in all the spectra in NIST 98 (released in 1998) have been fully evaluated (129,136 spectra, including 21,270 replicate spectra). NIST 98 was released with version 1.6d of the *MS Search Program* and then, version 1.7a in March of 2001. Even after the fully evaluated Database was released, spectra reported by users to have anomalies or to be of questionable quality were reevaluated. Generally, these spectra were replaced by carefully measured spectra if the compounds were available or they were removed if the reported problems were found to be valid. All new spectra procured or measured were submitted to the same validation process.

A good example of problems uncovered even after the complete evaluation is seen in Figure 1. Figure 1(A) is the NIST 08 EI mass spectrum from the \mainlib of 2-pentanone. Figure 1(B) is the first replicate spectrum for the same compound in NIST 08. Figure 1(C) is the spectrum of 3-pentanone. It is obvious that the spectrum in Figure 1(B) is of a mixture of the two compounds. The spectrum shown in Figure 1(B) which appeared in NIST 08 was removed from NIST 11. This spectrum had been accepted in error in the original evaluation process and had to be removed when the problem was exposed.

Beginning with the release of NIST 05 in 2005, GC method/retention index (RI) data and mass spectra obtained using MS/MS were distributed along with the EI spectra. The GC method/retention index data were mainly a compilation from the scientific literature. In addition to the reported RI and the detailed GC method [column type (packed or capillary)], dimensions (length and diameter), stationary phase and its loading (% for packed; film thickness for capillary), acquisition temperature (isothermal or programmed along with ramp rates and hold times), and mobile phase, type and flow rate) the literature citation is included with the title of the article. NIST 05 contained 121,112 records for 25,728 compounds measured on polar stationary phases with 12,433 compounds having spectra in the EI Database. NIST 08 contained 293,247 records of 44,008 compounds measured on both polar and nonpolar stationary phases with 21,940 compounds having spectra in the EI Database. NIST 11 contains 346,757 records of 70,835 compounds measured on polar and nonpolar stationary phases with 38,648 compounds having spectra in the EI Database.

NIST 05 had 5,191 spectra obtained of 1,920 ions using MS/MS techniques. NIST 08 had 14,802 spectra of 5,308 ions; and, NIST 11 contains 95,409 spectra of 12,568 ions. Spectra of both positive and negative ions were measured. Measurements were made using both tandem-in-time (quadrupole ion traps) and tandem-in-space (tandem quadrupoles and Q-TOF) instruments. Multiple collision energies were used and recorded. The MS/MS Database contains spectra for all the di- and tri-peptides. These spectra can be displayed in a graph format with peaks labeled as to m/z values or as to the type of ion (y and b), see Figure 2. When spectra acquired

using MS/MS techniques have m/z values greater than unity, the spectra are displayed with these accurate m/z values using the current version of the *MS Search Program*. Both the GC method/retention index (RI) and MS/MS databases are available standalone with *Search Programs* from distributors. The *Search Programs* provided with the standalone GC method/retention index (RI) database is substantially different from *MS Search* but has a similar user interface. This program offers a greater flexibility of interrogation of these data than is available with *MS Search*. The MS/MS standalone data is distributed with v.2.0g of the *MS Search Program*.

Part 2 of this article will be available later this month. Part 2 will look at the NIST Mass Spectral Search Program v.2.0g used with NIST 11.

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An Improved SPE/LC/MS/MS Platform for the Simultaneous Quantitation of Multiple Amyloid beta Peptides in Cerebrospinal Fluid for Preclinical or Biomarker Discovery **Company: Waters**

A previous application note described in detail the development of a fast, flexible SPE/LC/MS/MS platform for the quantification of multiple amyloid beta peptides from human or monkey CSF for the use in biomarker or preclinical discovery setting. In this note, the mass spectrometry platform has been updated from the Xevo TQ MS to the Xevo TQ-S system. This change facilitated both a 4X reduction in required sample size and a 4-5X increase in assay sensitivity.



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A method for analysis of 258 pesticides has been developed on Scion TQ triple quadrupole mass spectrometer. The method setup is streamlined with the new software approach of Compound Based Scanning (CBS). The matrix-matched calibration results have demonstrated good sensitivity, linearity and precision of Scion TQ.



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Developing New Methods for Pesticides in Dietary Supplements **Company: Restek**

New requirements for dietary supplements to be manufactured under cGMP regulations have created a need for methods to detect pesticides in these complex, largely botanical products. QuEChERS offers a simple, cost-effective approach that can reduce matrix interferences as well as variation among technicians. Here we demonstrate a procedure that incorporates a QuEChERS extraction, cSPE cleanup and GCTOFMS, resulting in good recoveries for a wide range of pesticide chemistries in dandelion root powder.





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Analysis of Cholesterol Lowering Drugs (Statins) Using Dried Matrix Spots Technology

Company: Agilent Technologies

Dried blood spot (DBS) technology combined with the analytical capability of modern mass spectrometers (LC-MS/MS) has recently emerged as an important method for the quantitative bioanalysis of small molecules. It is increasingly being looked at as a microsampling approach for preclinical and clinical pharmacokinetic/toxicokinetic studies. The primary advantage of DBS is the significant reduction in blood volume requirements, leading to cost and ethical benefits.



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Non-Targeted Screening of Lipophilic Marine Biotoxins by Liquid Chromatography - High-Resolution Mass Spectrometry

Company: Thermo Fisher Scientific

Marine biotoxins are produced by naturally occurring microalgae, whose populations can increase significantly under certain environmental conditions to form a harmful algal bloom. This report describes the use of the Thermo Scientific Exactive benchtop LC/MS system powered by Orbitrap technology for screening lipophilic marine biotoxins commonly found in shellfish. The method was optimized using a standard mixture of marine biotoxins, and then applied to a mussel tissue extract.



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Analysis of Phthalate Esters in Children's Toys Using GC-MS

Company: Shimadzu

As of February 2009, the US Consumer Product Safety Improvement Act, Section 108, mandated that all manufacturers of children's toys analyse their products for Phthalate Esters. The method to be used is in accordance with the US Consumer Product Safety Commission (CPSC). In this study, six phthalate esters in children's toys were measured using the GCMS-QP2010 Ultra. The simultaneous Scan/SIM data acquisition technique was utilized, with the SIM data used for quantitation. The data showed excellent calibration curve linearity and repeatability. In two of the toy samples, unregulated plasticizers were identified by scan data, illustrating the efficiency of simultaneous Scan/SIM measurement.

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