Applications of Computer Software for the Interpretation and Management of Mass Spectrometry Data in Pharmaceutical Science

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Abstract: The rapid growth of mass spectrometry (MS)-based computer software applications has been fueled by the unprecedented need to capture and analyze MS data and provide the information necessary for decision-making. Shorter timelines and a significantly greater number of samples has resulted in a tremendous focus on streamlined approaches that provide scientists, managers, and executives the capability to readily obtain, or even request, the necessary information that leads to accelerated product development. The generation of analytical data using roboticized high-throughput hardware has produced a bottleneck since data can be generated faster than it can be analyzed. New techniques including MS/MS and accurate mass experiments are feasible only using computers to capture and manage the enormous amounts of data necessary to perform the experiments. Whatever the nature of the experiments conducted, the MS analysis strategy is to extract the appropriate information required for decision-making in as facile a manner as possible. We will review here a survey of the creation of commercial and laboratory specific reference databases and associated searching algorithms and also recent efforts to introduce advanced processing and analysis algorithms to the hands of the masses, specifically as an aid to structure elucidation.

INTRODUCTION

Nine strategies (standard methods, template structure identification, databases, screening, integration, miniaturization, parallel processing, visualization, automation) consistently appear in MS-based methods for accelerated development [1]. These strategies serve to define the attributes of the analytical methods applied. High-throughput sample-generating technologies such as biomolecular screening and combinatorial chemistry can create many thousands of samples, each requiring the application of one or more forms of analytical chemistry. Nowadays, the ability to devise, construct, and refine sample-analysis methods, either chromatographic or spectroscopic, have become as equally important as the hardware itself. Today, the need to integrate appropriate method development strategies with MS processing capabilities is a critical factor in the modern industrial laboratory.

In chemical and pharmaceutical companies around the world, the necessity to acquire and analyze analytical data for the abundance of samples which abound today necessitates the availability of open-access laboratories containing highly roboticized instrumentation. The careers of professional spectrometrists are now focused on the implementation of optimal techniques to support the users of these laboratories rather than the standard sample analysis of yesteryear. Decreasing costs and reduced footprints for the instrumentation, as well as more intuitive software interfaces for non-specialists, allows the use of spectroscopic and chromatographic techniques in an open-access laboratory environment. Commonly, these laboratories provide NMR, MS, IR, UV-vis and chromatographic instrumentation. As a result of these laboratories both standard and hyphenated MS-based techniques have entered the hands of the masses. It is clear that there are distinct differences between the applications of mass spectrometry made available to non-specialists and that performed by the specialist. In general, non-specialists have access to instrumentation that generates a parent ion mass in soft ionization mode and possibly some minor fragmentation. In applications that deal with combinatorial plate analysis, for example, the data generated includes a full high performance liquid chromatography-MS (LC-MS) run. The ionizing technique is "soft" and produces for each well in a plate both the parent ion and one or more chromatographic traces [Total Ion Current (TIC), Extracted Ion Current (XIC), Diode Array, Chemiluminesence Nitrogen Detector (CLND), Elastic Light Scattering Detection (ELSD) and others] to aid in the assay of materials in the sample. Meanwhile, the traditional spectrometrist is generally more focused on non-routine analyses which today include accurate mass determinations, selected reaction monitoring (SRM), and full-scan MS/MS or MS^n. Whether the application provides data for synthetic chemists or expert spectrometrists, computer software is an essential component of the analysis. Whether it is the application of advanced chemometric algorithms for noise-reduction, the association of structural fragments with mass spectral features, or the management and databasing of the derived information, computer software applications additional to those required for operation of the instrument have been developed to deliver these capabilities.

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DISTRIBUTION OF SPECTROMETRY DATA TO CHEMISTS

The new technology which is delivered at each new analytical instrumentation conference continues to make significant advances. Similar to standard computer platforms, the cost and size of MS instrumentation with the same capability continues to drop almost annually resulting in the proliferation of open-access MS labs supporting chemists in both single and multiple synthesis environments. Typically, the resulting data is pre-processed by the generating instrument and is provided to the chemist in a hardcopy format or as a spectral image requiring a vendor-specific viewer.

Both of these scenarios prohibit direct interaction with the spectral data. While in some cases this is preferablesince the data is locked from further manipulation, as is necessary in a regulated environment, in a research environment such barriers limit the value of further analysis. The expense of installing a copy of vendor software on the desktop of every non-specislist accessing the MS instrument precludes this as a solution. In general, such an approach is in fact overkill as most chemists simply want access to the final spectrum. Traditionally, vendor software provides sophisticated data reduction tools but limited structure attachment and reporting capability. An alternative resolution to this problem is the installation of a third-party desktop processing solution for accessing the data directly over a computer network, allowing the chemist to further manipulate the data and store the resulting spectra in a database for further reference. Such an approach offers additional capability since it is common for a facility to utilize a heterogeneous mix of hardware platforms whereby spectra are generated. With the capability to read multiple file formats in their raw form, the costs of operation and the efforts to generate data portability are reduced.

Delivery of MS data to the desktop is only the first step within the data analysis puzzle for chemists. If the requirements are simply the distribution of the spectrum in an intuitive interface to replace the paper copy generated at the spectrometer, then the user would simply view the file as a simple stick-plot (Figure **1**). The common approach adopted by the chemist is to correlate the expected molecular formula with the parent ion mass in order to be able to declare the spectrum consistent or not. Generally, this approach involves the use of a calculator to determine the expected mass or, as with some versions of vendor software, the declaration upfront regarding the expected molecular formula and the confirmation by the package that such an ion is determined in the spectrum. A more complete analysis allows the chemist to use the actual molecular formula to predict the isotope pattern for the molecular formula and compare with that obtained experimentally. Such an isotope pattern is generated simply by inputting the chemical formula into a spectrum calculator (see Figure **2**). These calculators allow the calculation of nominal, monoisotopic and average masses.

At many stages of a synthesis, but specifically at the final stages, chemists require additional data generated within within the open-access facility. Chemical structures are commonly confirmed using a combination of techniques

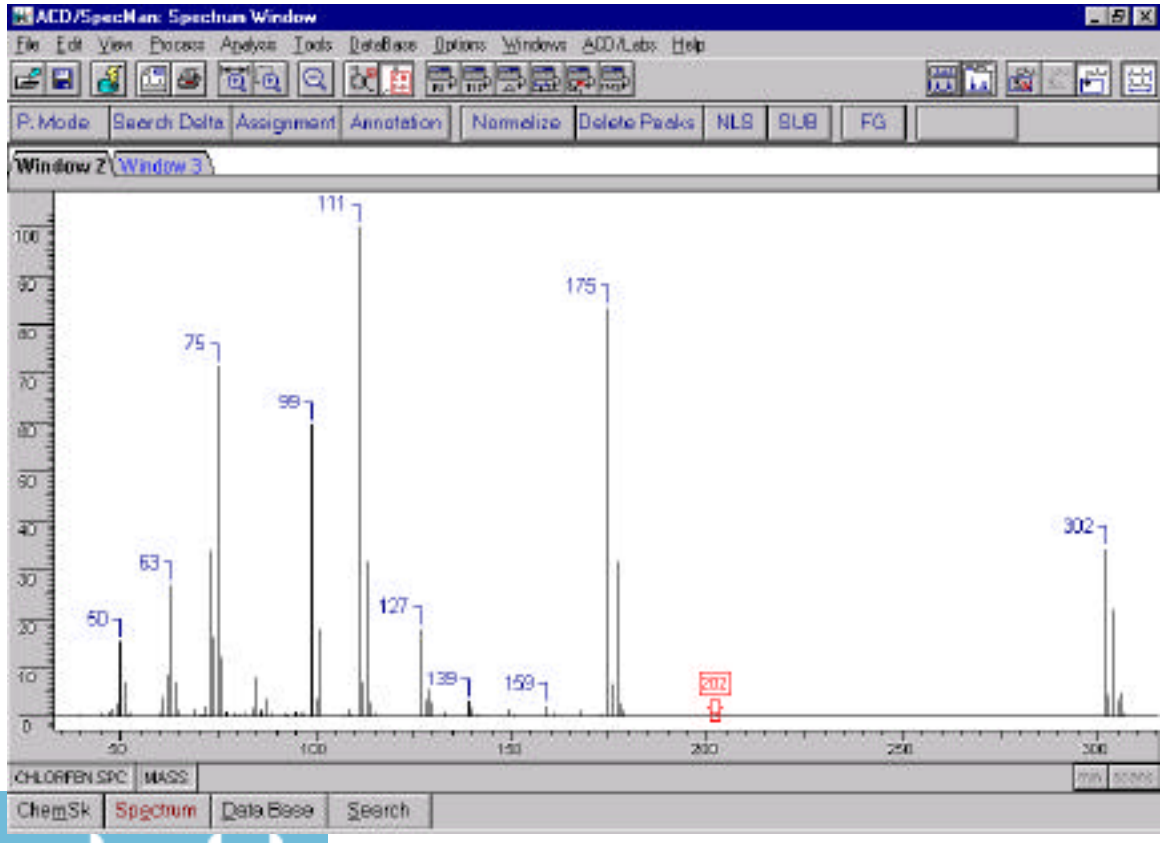

Fig. (1). The Electron Impact (EI) spectrum for a sample expected to be 4-chlorophenyl 4-chlorobenzenesulfonate.

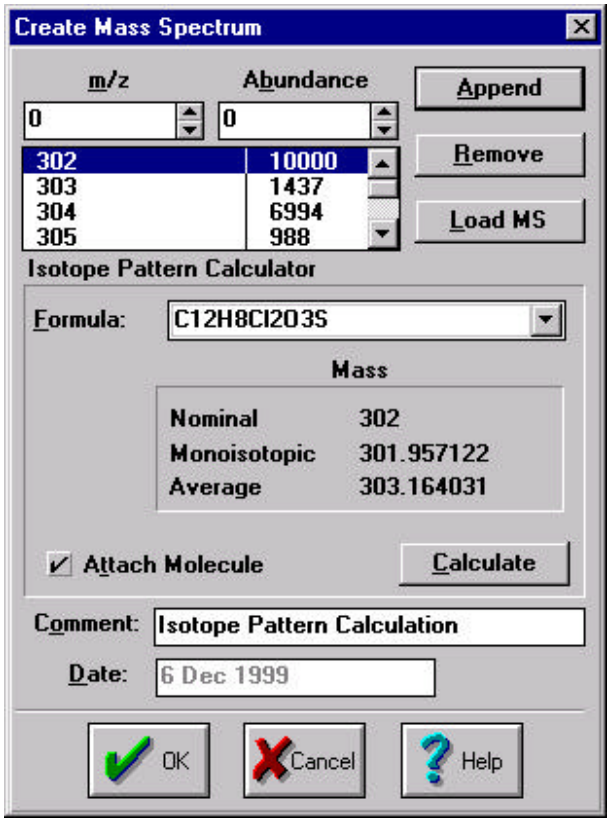

Fig. (2). An Isotope Pattern Calculator showing Nominal, Average and Monoisotopic masses.

such as Nuclear Magnetic Resonance (NMR) and IR in association with the MS spectum. In this case, samples are often distributed to the relevant laboratories for the generation of a more complete data set. This procedure can include the application of LC-MS, collisionally induced dissociation (CID) and MS^n. When these data are generated, the analysis commonly becomes the responsibility of the spectrometrist rather than the chemist. The reasons for this include the fact that multiple forms of spectroscopy data cannot be processed and viewed in a single integrated software package. Appropriate tools are now available which greatly enhance the throughput of structure elucidation problems through the MS laboratory and hand over partial responsibility to the chemist. These tools include LC-MS data handling, noise reduction, fragment assignment and fragmentation prediction. Each of these will be examined in further detail below in terms of spectrometric tools; but specifically the emphasis is that the provision of such tools to chemists could facilitate both data handling and analysis.

DESKTOP DATA ANALYSIS AND TOOLS FOR MS SPECTROMETRISTS

Chemists generating MS data are generally non-specialists and require a simple way to access, process and visualize data files at their desktops. One added complexity is that today many analytical laboratories contain spectrometers from multiple vendors. Each vendor delivers instrument-specific data generation and processing capabilities. In general, these capabilities are inherently similar. Instruments are distinguished primarily on performance, ease-of-use and cost. There is no doubting the influence which the software interface and capabilities can have in deciding which instruments are installed in any

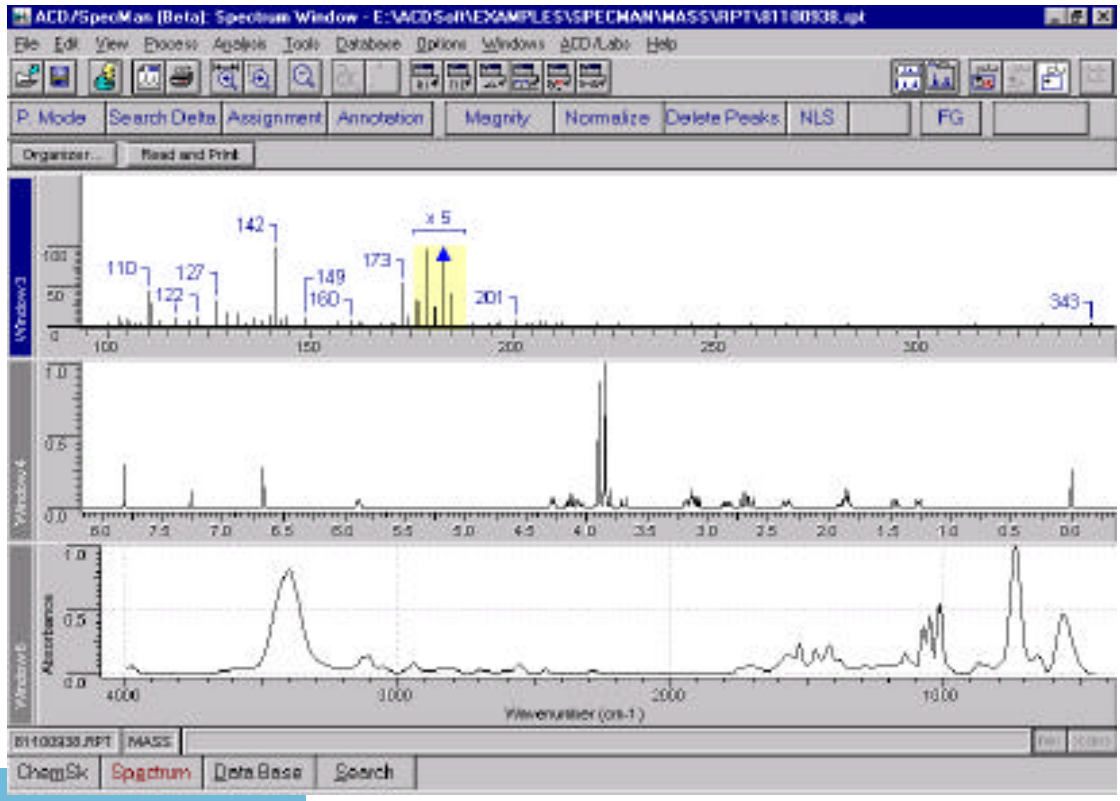

Fig. (3). A multi-window display of MS (top), NMR (middle) and IR (bottom) spectra. This ability allows unified desktop viewing of data.

particular laboratory. Indeed, in many cases the software can play a dominant role in the decision-making process.

Any particular analysis may require the acquisition of data from one or more of these instruments. Due to the heterogeneous instrumentation environment and resulting distribution of data formats, it is almost impossible to bring together a single universally applied interface the data. The ability to read in raw vendor formats and allow integrated data-handling has been severely lacking. Efforts have been made to define common exchange data formats such as JCAMP and NetCDF. Third party vendors have also [2] assumed the task of becoming the neutral party to unify data handling and management. Such third party offerings have become a crucial component in the effort to build a single corporate spectroscopic database supporting all instrumentation, not limited to MS but inclusive of NMR, IR, UV-Vis, Raman and HPLC as shown in Figure **3**.

There are numerous technologies for generating data at the disposal of the mass spectrometrist, and it is impossible to be exhaustive here. A number of the various forms of MS experimentation have been addressed earlier in this volume. Standard capabilities for the handling of LC-MS data should be available within all MS processing software: generation of ion chromatograms, spectral subtraction of mass chromatograms, averaging of spectra, combining of spectra, baseline correction, peak picking and integration. These processing tools are particularly useful for the identification (or classification) of unknown structures where authentic standards are often not available.

In the pharmaceutical industry, the MS-based identification strategy is based on the premise that much of the parent drug structure will be retained in the metabolites, impurities, or degradants [3]. The resulting fragment ions associated with unique substructures of the parent compound would also be (expected) retained. Direct comparison of molecular weight and fragment ions using the ACD MS processing and analysis toolkit reveal substructural differences and lead to an interpreted or proposed structure. Thus, the unique fragment ions contained in either full scan or product ion mass spectra of the parent compound serve as the *template* for identification. The template structure identification strategy has been recently illustrated for the profiling of paclitaxel degradants [4]. While MS vendors are astute at providing tools for analysis, they have not serviced the requirement of providing chemical structure integration tools to aid in this analysis.

Detailed information is obtained by the observation of sequential neutral losses to determine the sequence of substructures or "molecular connectivity" within the analyte [5]. This procedure is analogous to two-dimensional NMR techniques used to sequentially connect substructures. Of course, a familiar example of molecular connectivity is the

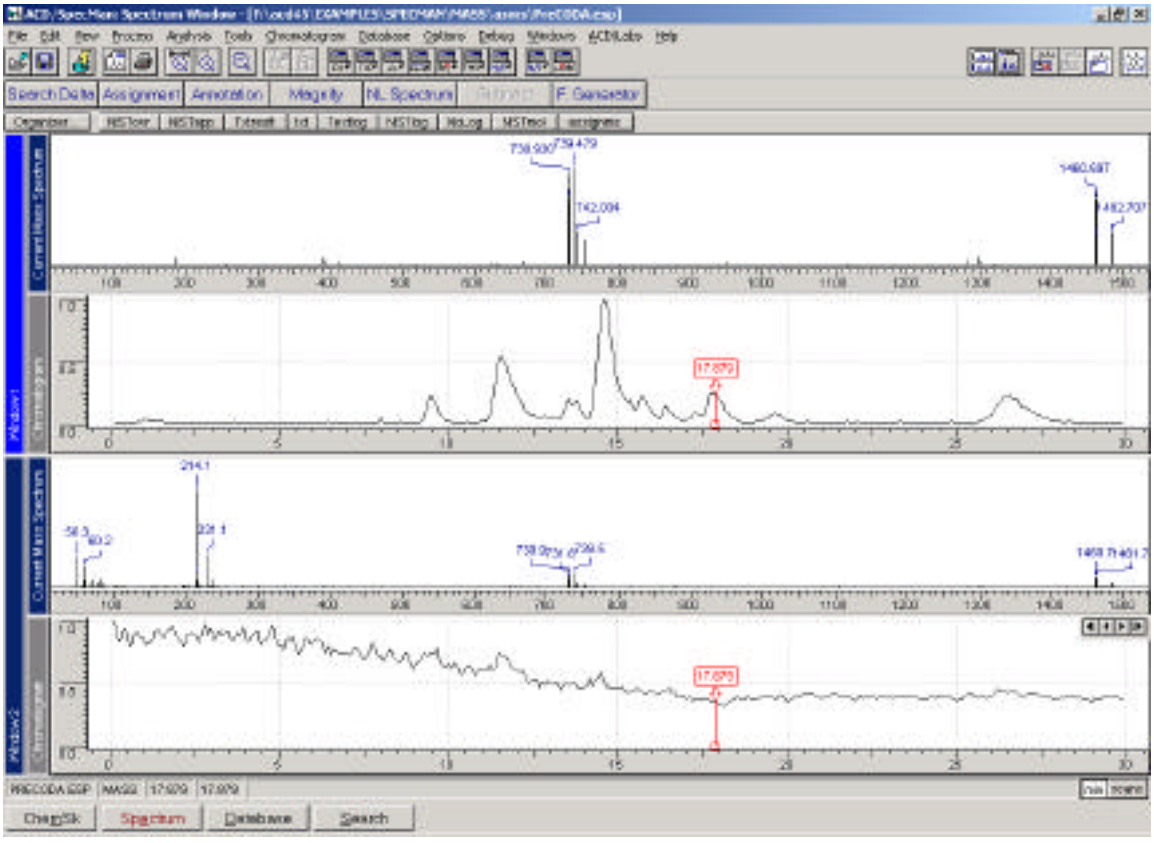

Fig. (4). A 340 scan LC-MS spectrum containing multiple metabolites. Notice the high levels of noise and background in the total ion chromatogram (lower panel). The mass spectrum in the top window is for the scan at a retention time of 17.8 minutes. Notice the low molecular weight components around m/z 214. Notice the removal of the gradient background after application of the CODA chemometrics algorithm and the significant decrease in noise level in the Total Ion Chromatogram.

determination of the amino acid sequence of a peptide. Specific neutral losses are indicative of certain amino acids, and the sequence of these losses identifies the peptide [6].

Furthermore, application of such tools to an LC-MS run of the nature displayed above would be difficult without capabilities to reduce the noise within the data. Inherent to the data handling tools are noise reduction algorithms (e.g, Biller-Biemann). Recently, the CODA algorithm (COmponent Detection Algorithm) reported by Windig *et al.* [7] has been integrated into MS processing software in order to produce dramatically improved noise reduction. Figure **4** shows an example of the results which advanced chemometrics algorithms can deliver. Shown in the figure is the LC-MS run before and after the application of the CODA algorithm.

Using a suggested chemical structure for a particular spectrum, which is a common approach for structure verification and identification, the spectrum can be analyzed in terms of hypothetical fragmentation. One avenue is to use a "*lasso tool*" to highlight molecular fragments that will indicate the fragment within the spectrum (Figure **5**). This capability is presently delivered by third party software tools [2a]. Following the import of a mass spectrum, a chemical structure is attached using the molecular structure editor integrated into the program. The lasso tool is used to encircle a particular fragment, and if a signal corresponding to its mass exists in the spectrum, the fragment is highlighted and the assignment is added to the assignment table. In this way, an entire mass spectrum can be assigned and examined for consistency with the hypothetical structure. If there is a mixture of components in a single spectrum due to co-elution, then each component can be individually assigned.

An alternative approach to aid in the identification of an unknown is to perform a spectrum or subspectrum search against a database of known structures and associated spectra. A simple search based on just a few peaks from the mass spectrum is possible. McLafferty developed two search techniques, based on the probability of certain ions (PBM) as well as a technique based on a collection of chemicals fragments associated with certain fragmentation patterns [8].

Over the years collections of mass spectra have been collected by different groups. The National Institutes of Health (NIH) and Environmental Protection Agency (EPA) standardized the data collection and analysis of the data to ensure a high quality aggregation of tens of thousands of spectra. In addition, Stenhagen, Abrahamsson, and McLafferty collected thousands of mass spectra to form one of the standard reference databases available today. The standard computer readable collections are those of the US Government, distributed by NIST and the McLafferty collection [9]. The NIST collection has a quality index associated with each spectrum based on particular evaluation criteria.

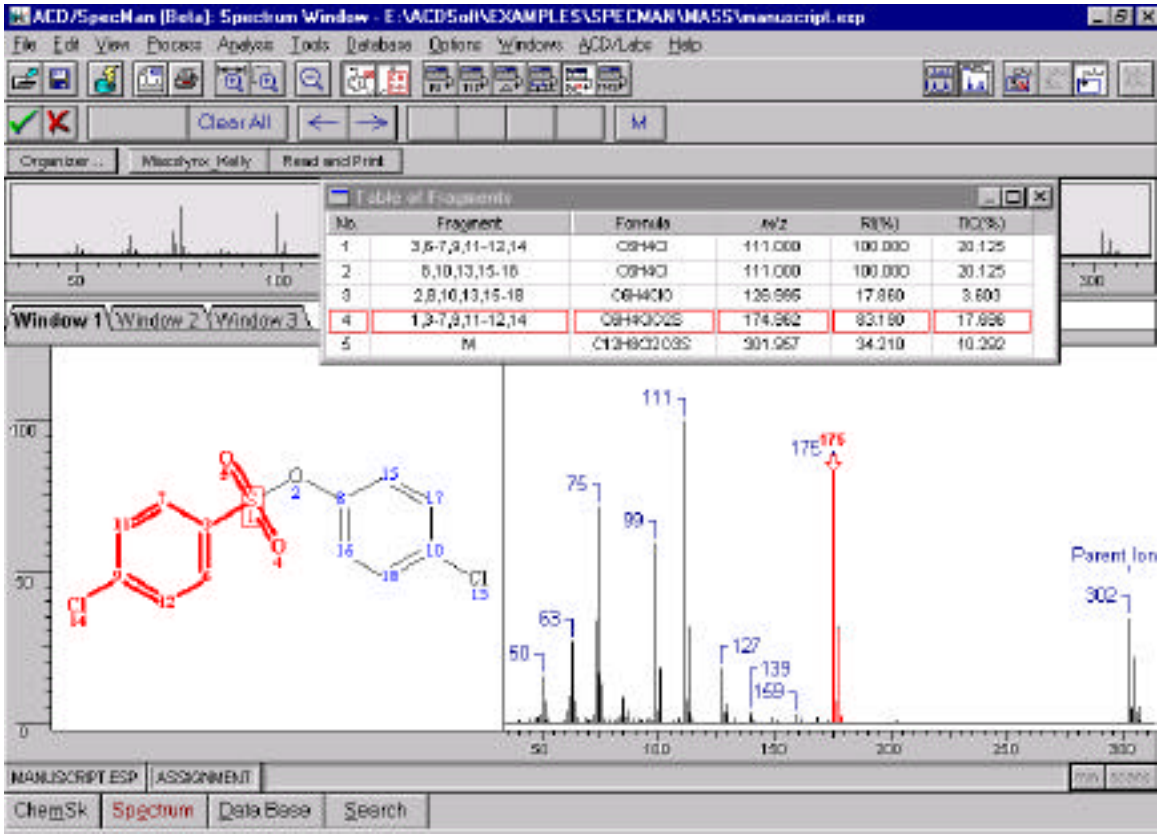

Fig. (5). Assignment of the spectrum of 4-chlorophenyl 4-chlorobenzenesulfonate using the "lasso tool". The fragment table lists assigned fragments. Moving the mouse cursor over the table highlights the assigned molecular fragment in red on both the spectrum and the structure.

The categorization of processed information into databases is a powerful approach for leveraging the advantages of high throughput analysis schemes. Although a modest amount of time is required to implement this strategy, databases have two important benefits. Firstly, it provides a reference-friendly format to search data and this feature is essential for the rapid identification of known compounds. For example, the identification of a metabolite structure may require only a retention time and molecular weight information via LC-MS analysis when compared to the metabolite structure database compiled from previous studies [10]. A second benefit of databases is the efficient extraction of information. Databases may be "mined" to detect trends that may not otherwise be noticed. This approach can be used to reveal trends such as the metabolically active sites of a molecule and/or substructures labile to degradative conditions, for example.

Once created, a database may be transferred to other laboratories and facilities that are participating in a particular research activity. The resulting databases can be distributed via standard server technologies or "web-enabled" and made accessible via corporate intranets or public internets. Information is coordinated within the database, and a variety of scientists are able to effectively pool and merge their information. When implemented early within the product development cycle, valuable information for later stages in drug development is available [11]. Therefore, this approach provides a comprehensive method for information gathering whereby future projects are planned, coordinated, and efficiently supported.

It is worthwhile noting that database creation, modification, and use benefits greatly from a standard, systematic method. This approach produces reliable datasets that lend themselves to a highly consistent database format throughout a project lifetime. While spectral databases can be purchased these are generally limited to nominal mass EI data. Since library searching techniques are limited by the size and nature of the library, relative to the particular problem of the chemist the creation of user databases are of high value to any corporation. With today's technologies allowing the generation of chemical ionization and accurate mass data in-house databases can certainly be of significantly higher value than commercial databases. The searches of such databases can be defined according to a series of options and multiple databases can be searched simultaneously. For the chlorobenzenesulfonate case above (shown in Figure **5**), the whole spectrum was searched against a total of over 130,000 spectra distributed across three databases to generate 5 hits (Figure **6**).

Search efficiency is increased by imposing constraints. As an example of a multi-step constrained search approach, a search of the NIST database for a para-substituted benzene sulfonic acid fragment, as a starting point, gives a total of almost 300 such spectra in the database. This subset of spectra can then be searched according to variables such as

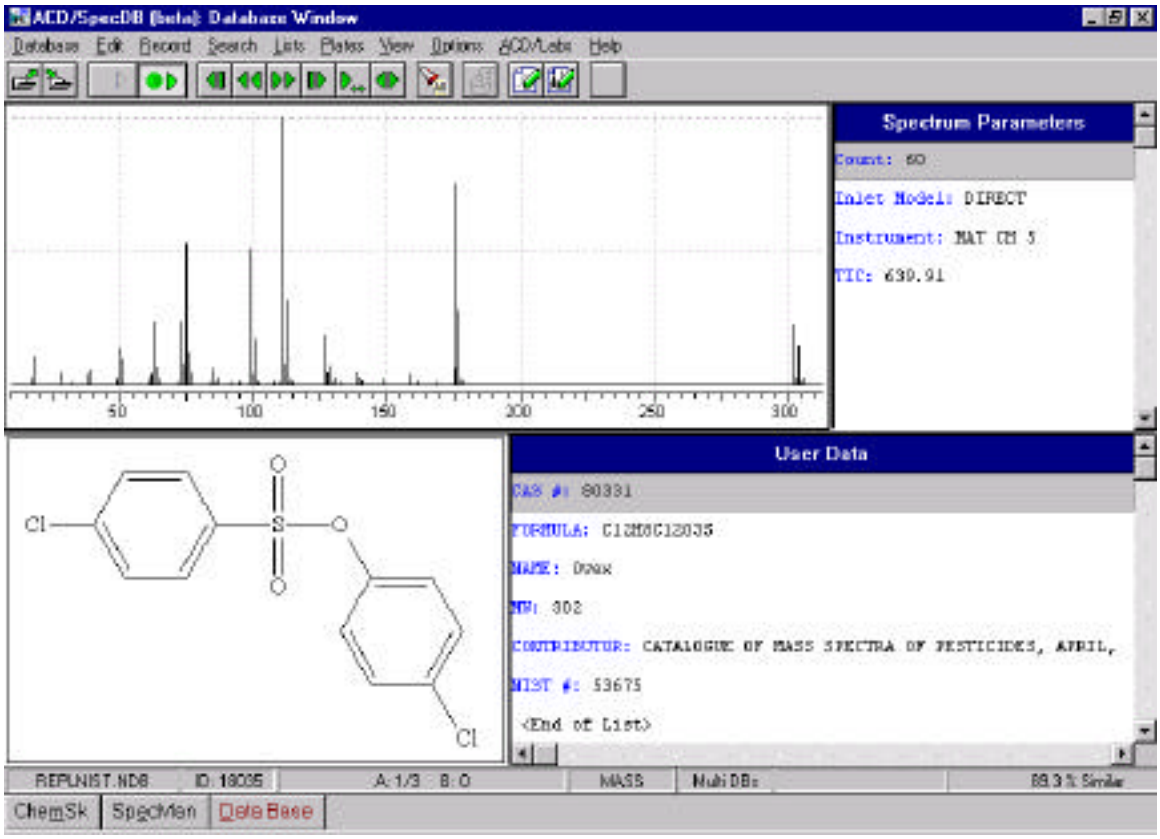

Fig. (6). The most similar match (89.3% match factor – see bottom right hand side) for the spectral search displays the spectrum of Ovex, from the catalogue of mass spectra of pesticides from within the NIST replicates database. The structure of Ovex is consistent with the suggested structure.

molecular formula, elemental composition based on elemental analysis, and substructural components based on identified fragmentations (loss of Ph, CCl_3, $C(CH_3)_3$ and so on).

Often, when work is initiated on new project compounds, the use of a complete spectral database is not possible (i.e. drug discovery). When information is stored within a comparative database, compounds of interest can be effectively searched and identified for use in early to late stages of development [12]. Database capabilities also permit the use of substructure-based searches to identify compounds within a specific dataset or library that contains a distinct substructural entity [13].

Yet another approach is the ability to **predict** the fragmentation of a molecule using rules-based prediction and assuming knowledge of the hypothetical structure as discussed earlier for the actual fragment assignment (see Figure **7**). For the chlorobenzenesulfonate molecule example discussed here, the fragmentation tree and specific fragments are shown. Selection of each branch in the fragmentation tree displays the associated ion that can be compared to those displayed in the spectrum window.

INTEGRATED SPECTROSCOPIC AND CHEMICAL STRUCTURE DATABASING

Integration strategies often encompass separate events involving instrumentation, methodology, and process. Conventional methods of analysis involve multiple steps. For example, the identification of natural products traditionally involves the scale-up of fermentation broths, solvent extraction, liquid/liquid or column fractionation, chromatographic fraction collection, and spectroscopic analysis of the individual components. The integration of these bench-scale steps into dedicated systems provides unique and powerful advantages for on-line, and perhaps, real-time analysis [13]. Arguably the most significant bottleneck that exists in industry today is the ability to integrate these traditional analysis steps with MS processing and analysis.

Discovery chemists and the research and development environments focus a lot of effort into the resolution of components with direct attention paid to the actual chemical structures. As a result, for spectroscopic techniques such as NMR, MS and IR, it is common to find filing cabinets full of spectra, relevant scientific literature, and associated information, generally linked to the chemical structures that gave rise to the specta. Even though electronic libraries of chemical structures and MS spectra exist, these libraries are usually limited to EI data as discussed previously. It is possible to search experimental MS data against these libraries with the intention to aid in the identification of possible unknowns. These libraries are, however, NOT structure or substructure searchable. The requirement for the electronic management of experimental spectra with associated chemical structures is an obvious requirement. There are two general forms to such databases. For *spectral-centric solutions* the primary focus of the software is the

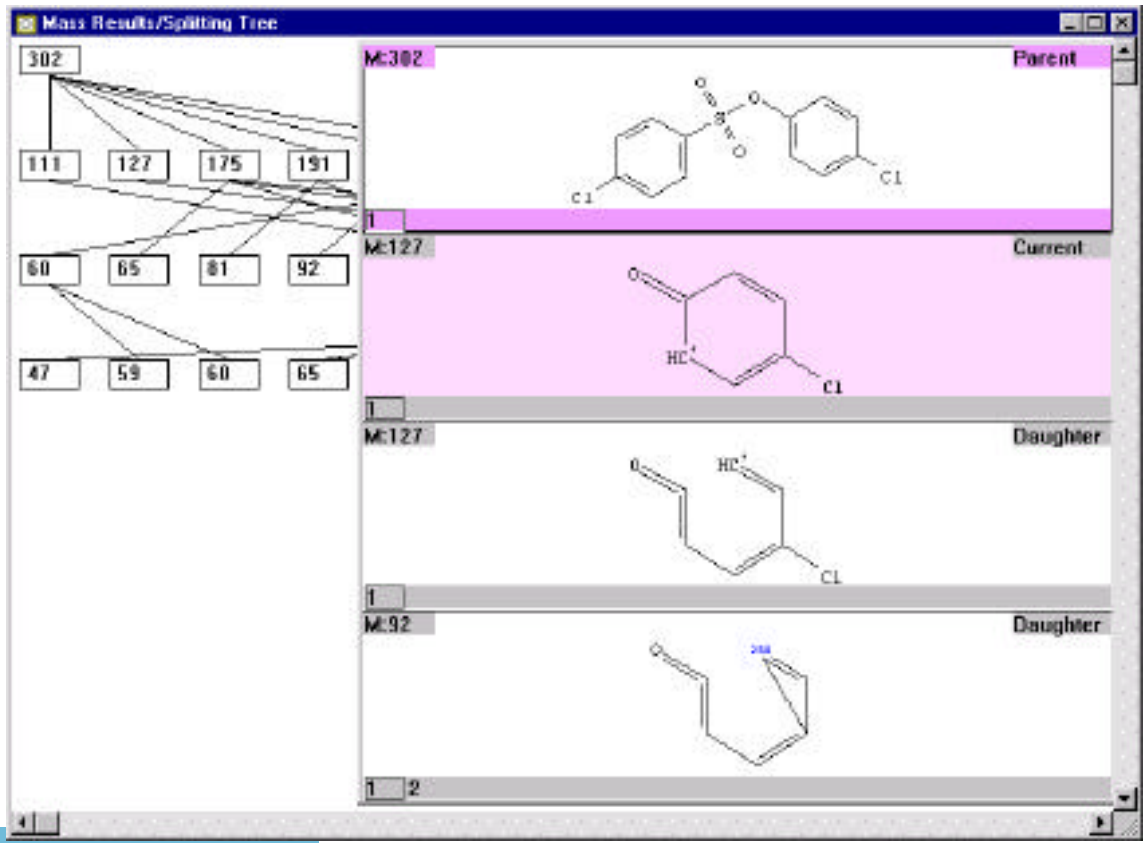

Fig. (7). An expansion of the predicted fragmentation tree for mass 302, the parent ion, and possible daughter ions resulting from fragmentation through m/z 127.

desktop processing of spectroscopic data, followed by the concomitant association with chemical structures. Commonly, a particular facility has access to a structure databasing system from one of the multiple vendors providing this type of solution. These structure databasing systems provide a *structure-centric solution* whereby spectral records are attached to the structure records in the database for viewing and further processing.

Spectrometrists and chromatographers utilize a variety of technologies to both separate and identify chemical structures. It is common in today's analytical environment to find teams assembled with skillsets to generate both optimal separation and analysis solutions. Spectrometrists assign their spectra in relation to chemical structures using parent ion mass or fragment ion mass analysis in MS, nucleus to peak assignments in NMR and vibrational bands to IR peaks, for example. Spectrometrists have used the standard filing system of drawers full of spectra with an association of the file number with some textual identifier in order to locate the detailed knowledge extracted from the spectra at a later date. The general level of spectral management has been limited to hand written notes in notebooks or sometimes text-searchable databases pointing to associated spectra.

Tools are now available to allow spectra to be databased in electronic format with associated chemical structures [2a]. In this manner, the mass spectrometrist now has the opportunity to search the database for related structures or substructures, or spectral features when performing fresh analyses. When integrated with other spectral data the result is a legacy database of multiple spectroscopy data, thereby building a foundation for future analyses. The value residing in such tools is the time-savings that result for the analysis of related chemicals and the exchange of information between different analytical laboratories within the same company. In theory, such an approach should not be isolated to spectrometry; for chromatography, tools now exist to allow the similar integration of chromatographic peaks and chemical structures.

Resulting spectra with associated chemical structure(s) carry valuable information for future analyses. Such resulting files can be stored on a centralized server and thus become a powerful means for dissemination of the mass spectrum-structure connectivity and fragment assignment information. This general approach can be expanded to a world wide web intranet approach whereby the spectra are posted as individual HTML pages with hyperlinked MS files.

Software solutions available today allow each spectrum to be databased with associated chemical structures, thereby offering significantly enhanced capabilities over the common file systems used today in many laboratories. Due to recent advances in database technology there is enhanced searching capability over the standard filing cabinet system or a text-based databasing system. It is possible to search the resulting databases by structure, substructure, formula, molecular weight, chromatographic and spectroscopic parameters or user data. User data includes the creation of user-definable database fields with particular field labels including, for example, submitter, project name and type of analysis, all of which become searchable fields. Multiple

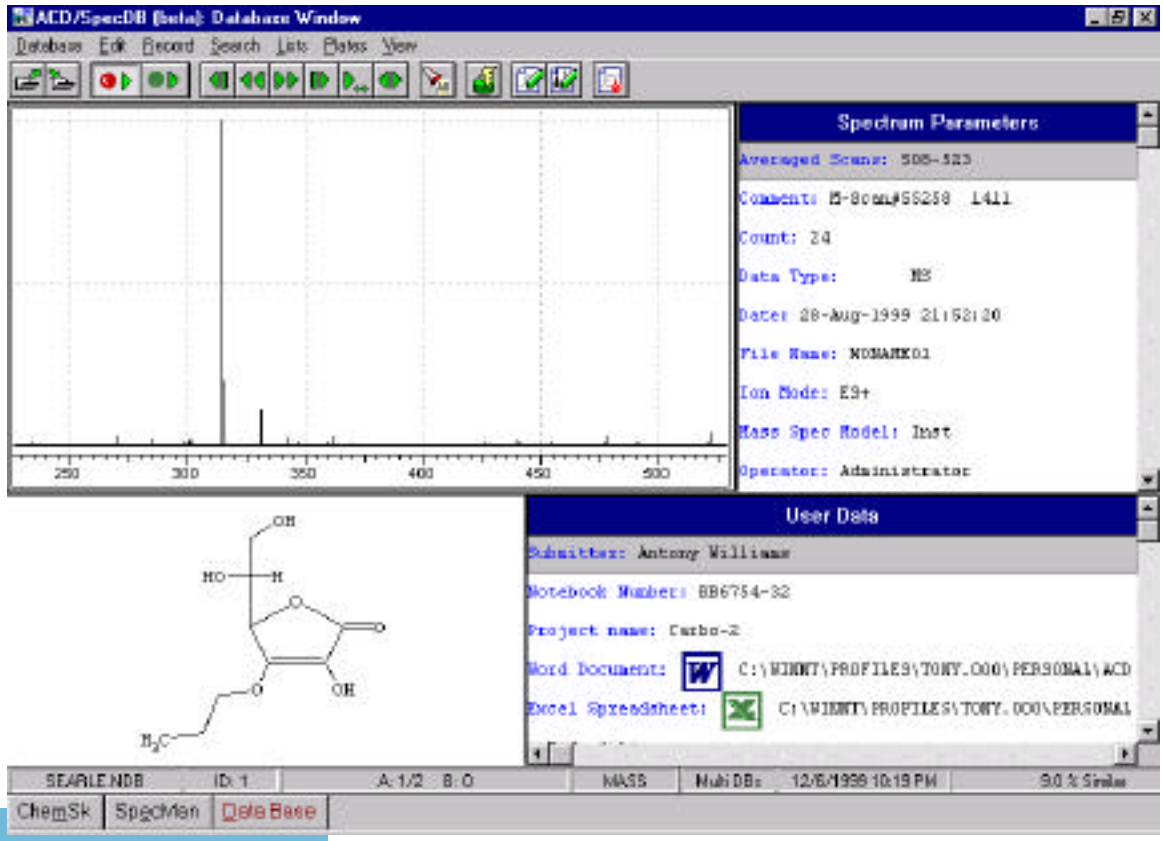

Fig. (8). Construction of an in-house database including spectrum, structure(s), spectral parameters and links to reports and spreadsheets. Note the links in the user data fields to the Word reports and Excel tables.

databases can be searched at one time, thereby allowing different databases to be constructed according to analysis type, project name, individual user and so on. These multiple databases can also be distributed *across* different departments, divisions or even an entire corporation, simply by using the ability to point to databases located on mapped network drives. Corporate-wide database capability engenders concern about the integrity of the databases. This can be addressed by standard database security features.

Other than the spectrum parameters, the association of individual searchable user data fields is invaluable, thereby allowing each spectrum in the database to be associated with a project, a customer, an analyst or any other appropriate information (Figure **8**).

The value of the approach outlined here should be obvious as the ability to integrate structural information with spectra into a database offers exciting benefits to the spectrometrist and is an ideal solution for an environment where multiple spectrometrists need to quickly determine assignments and identify specific chemical structure classes. The additional benefit of this tool is that it is also fully integrated with a similar toolset for spectroscopy allowing similar structure-spectrum management for NMR, MS, UV-Vis, IR and Raman.

CONCLUSIONS AND FUTURE PROSPECTS

Computer software technologies for the processing and analysis of MS data and the management of the resulting knowledge are quickly emerging. While it is almost impossible to define the long term future of MS data processing and analysis, it is likely that MS data will be acquired with even higher mass resolution and the tools necessary to manage this data will need to be further developed. With higher mass resolution comes unambiguous molecular formula identification. However, only MS fragment analysis or integrated data analysis with other forms of spectroscopy will provide further structural detail. While LC-NMR-MS instrumentation is already available, it is likely that such extended hyphenated technology will become more common. Software tools allowing integrated data handling, management and prediction for these multiple spectroscopies will be made available. The tools which will be delivered in the future will have to include additional developments in the area of highly automated processing of thousands of datasets, advances in MS fragmentation and tools for the creation and searching of accurate mass spectral databases. Such an approach, when further integrated with spectral processing and databasing for other techniques (NMR, IR, UV-Vis etc.) will provide a unifying tool for spectroscopy management.

With further research into statistical and chemometric methods it is hoped that further techniques will be developed for mass spectral identification. However, MS, in any of the separate ionization techniques (El, CI, FAB, ED and so forth) has inherent limitations. Only in the presence of additional techniques, such as IR and NMR, will structure elucidation and verification be more rigorous when identifying the structure of unknown chemicals.

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