Metabolite Identification Using ACD Labs Version 8.0 Software and the Agilent 1100 LC/MSD Trap XCT Plus System

Technical Overview

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Introduction

Metabolism is the very essence of life. An organism ingests substances, transforms them into intermediates, extracts useful energy or compounds, and excretes the remainder. The intermediates and excreted materials, metabolic products, contain a wealth of information about the genotype and health of the organism.

Drugs are a specific class of these substances, usually administered to an individual for a given effect, to relieve pain, reduce a headache, prevent infection, and so on. They are metabolized just like all organic substances to produce a substantial array of products. Some of these metabolites may be even more potent than the original drug; others are quite toxic to the individual. Different individuals can metabolize the same drug quite differently, at substantially different rates and with vastly differing array of products. Prior to release of a drug candidate for general use, it is necessary to know what metabolites are formed, how they are distributed in the organism, what forms are excreted, at what rate the drug is metabolized, at what rate the metabolites are excreted, and so forth.

A major task of drug development is the identification of the metabolites of drug candidates. Due to the large number of metabolites that are formed, their structural diversity, and their relatively low concentrations, a sensitive analytical technique and sophisticated software are needed for comprehensive analysis of samples from metabolism studies. The pairing of an LC/MSD ion trap mass spectrometer and software for chemical and structural interpretation of the mass spectral data is an excellent combination for this type of analysis.

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Software, instrument, and samples

The software represented here is ACD Labs software 8.00 release, MS Manager Suite. It consists of several modules, including MS Manager, ChroManager, UVIR Manager, MS Fragmenter, ChemSketch, SpecDB, ChemFolder, ChemBasic, HNMR Viewer, and CNMR Viewer. The installation program also installs numerous guides and reference manuals for these modules. Frequent updates are available via the Internet.

The instrument used to acquire the data was an Agilent LC/MS system consisting of Agilent 1100 Series solvent degasser, binary pump, well plate sampler, and thermostatted column compartment, and an Agilent 1100 Series LC/MSD XCT Plus ion trap mass spectrometer.

The data shown in this note resulted from the analysis of urine samples from a single volunteer. A control sample was taken, followed by ingestion of an over-the-counter drug of interest, dextromethorphan. Dextromethorphan is an antitussive used to suppress coughing, but it is also sometimes abused as a recreational drug. A pooled urine sample containing excreted drug metabolites was collected over the next several hours. The details of the sample preparation and instrumental analysis parameters will be the subject of a separate application note.

Results and Discussion

Data import from multiple instruments and manufacturers

A typical analytical laboratory which performs metabolism studies has a wide array of instrumentation: NMR, MS, IR, UV/Vis—sometimes several different types of each instrument and from different manufacturers. It becomes a bewildering challenge to obtain useful information from the variety of instruments, each with its own software conventions. The ACD Labs software comes with numerous import filters for many instrument types and manufacturers (see Figure 1). It has several interlinked modules that can process data from many instrument types. Thus, a single data system and software can process data for the entire lab. The savings in time and training can be considerable.

Automatic comparison of control and sample

A sample usually contains endogenous components from the normal metabolic processes as well as metabolites from the drug candidate. It can be a tedious process to sort through data looking for small differences between the sample and control. The ACD Labs software has a powerful COmponent Detection Algorithm (CODA) that dramatically reduces the noise in LC/MS data, allowing detection of minor components in a sample. After applying CODA to both the sample and

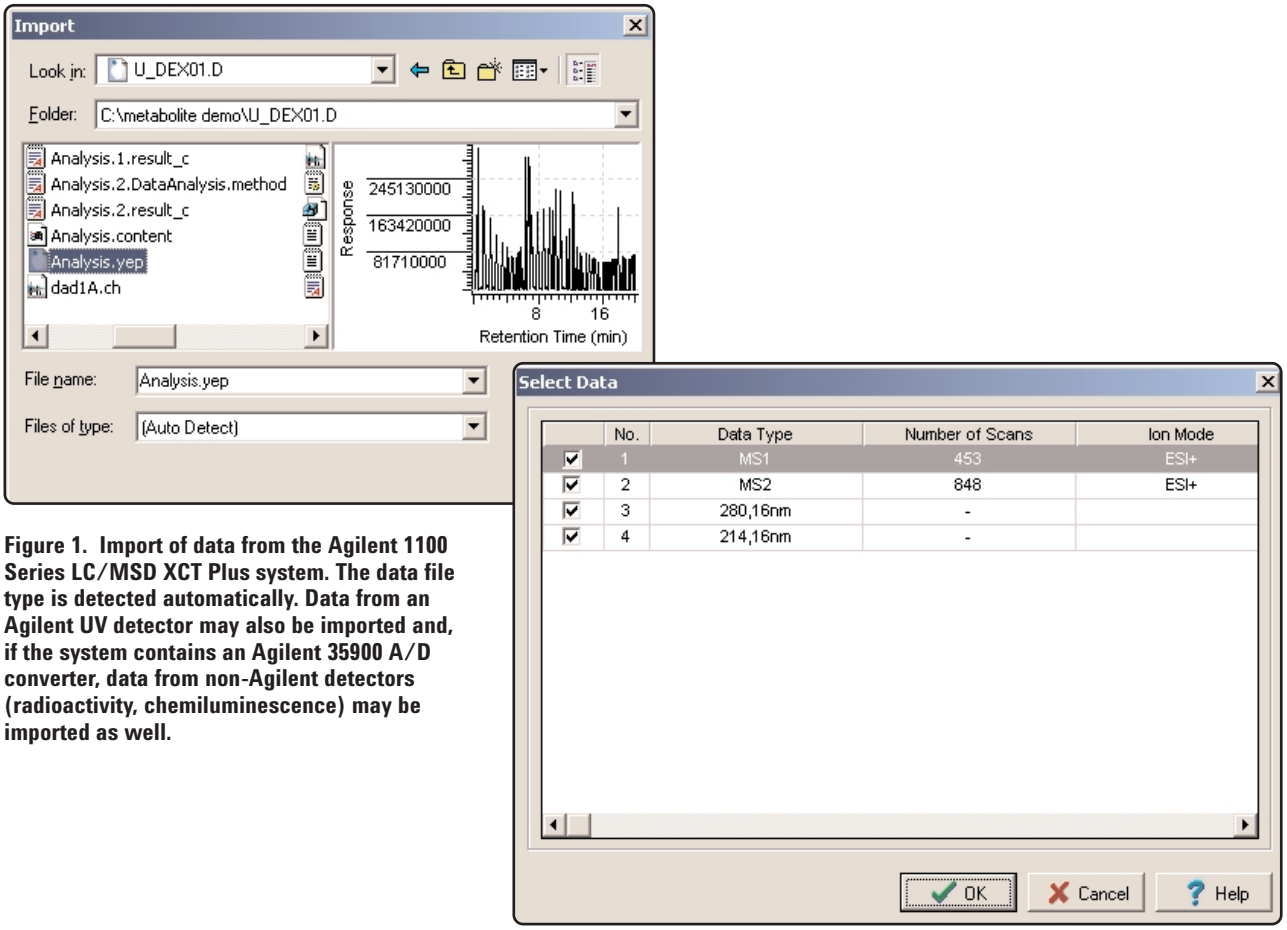

Figure 1. Import of data from the Agilent 1100 Series LC/MSD XCT Plus system. The data file type is detected automatically. Data from an Agilent UV detector may also be imported and, if the system contains an Agilent 35900 A/D converter, data from non-Agilent detectors (radioactivity, chemiluminescence) may be imported as well.

control, a second algorithm named “Compare LCMS” finds the unique differences between the mass chromatograms of the two data files. An example of this Compare LCMS processing is shown in Figure 2, where three peaks are clearly present in the colored mass chromatograms of the sample. These are the three major metabolites of dextromethorphan. Of equal interest is a comparison of the black TIC traces of the sample and the control. An endogenous compound eluted at 6.2 minutes in both analyses, yet the Compare LCMS operation did not produce any mass chromatograms that maximized at this time. In other words, the endogenous compound is not a significant difference. These processing tools may save considerable analysis time and allow the investigator to focus on the relevant data.

Easy structure drawing

Once the significant data in the sample has been identified, the next step is to create a hypothetical structure for the metabolite represented by that data. Drawing structures is a rapid process using the ChemSketch module (Figure 3). Most structures may be retrieved from the online dictionary of over 125,000 compounds. Modifying the retrieved structure to create the structure of a

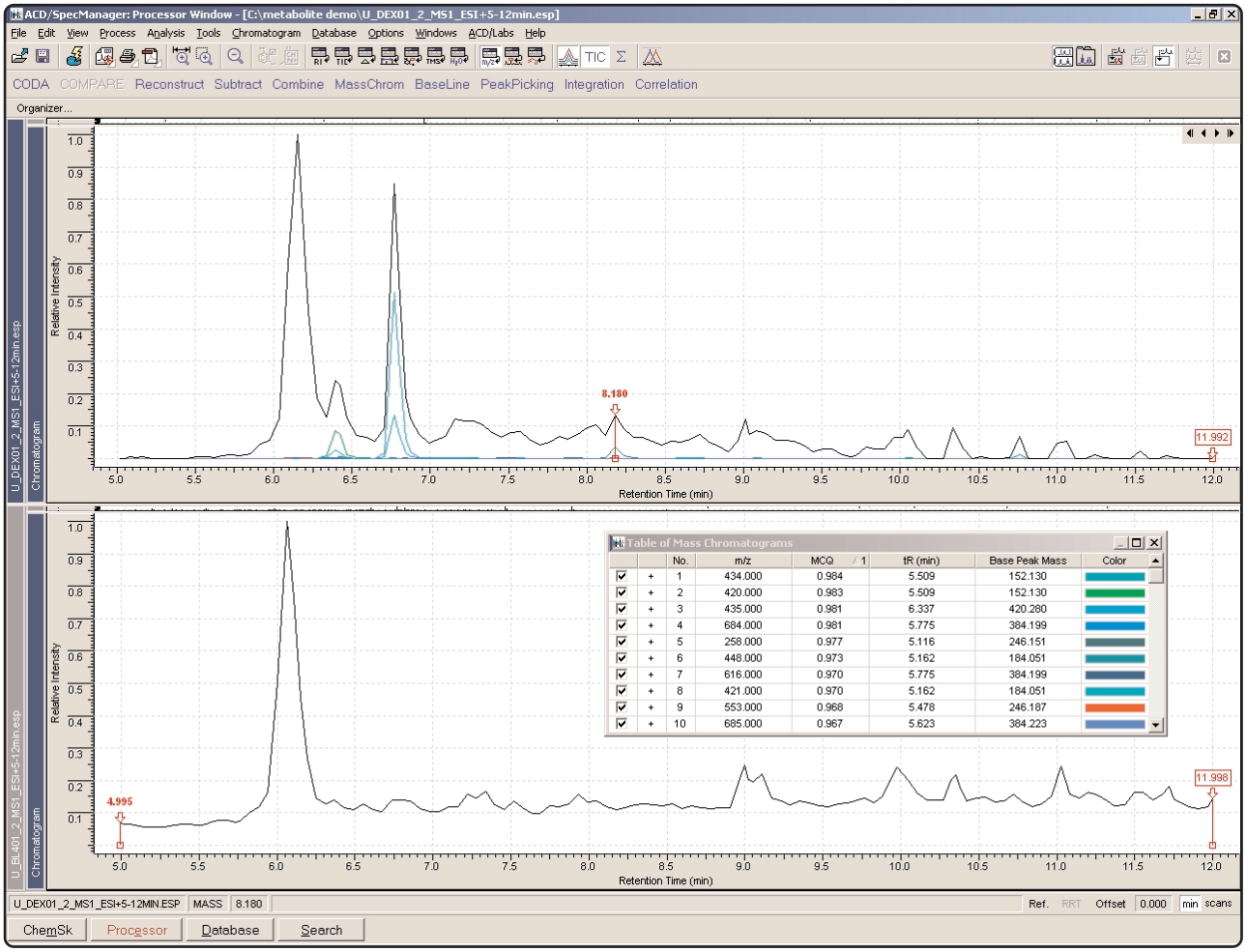

Figure 2. Compare LCMS processing results for sample (top) and control (bottom), showing three major dextromethorphan metabolites (colored traces). An endogenous compound eluting at 6.2 minutes in both analyses (black traces) is not considered a significant difference in the two data sets.

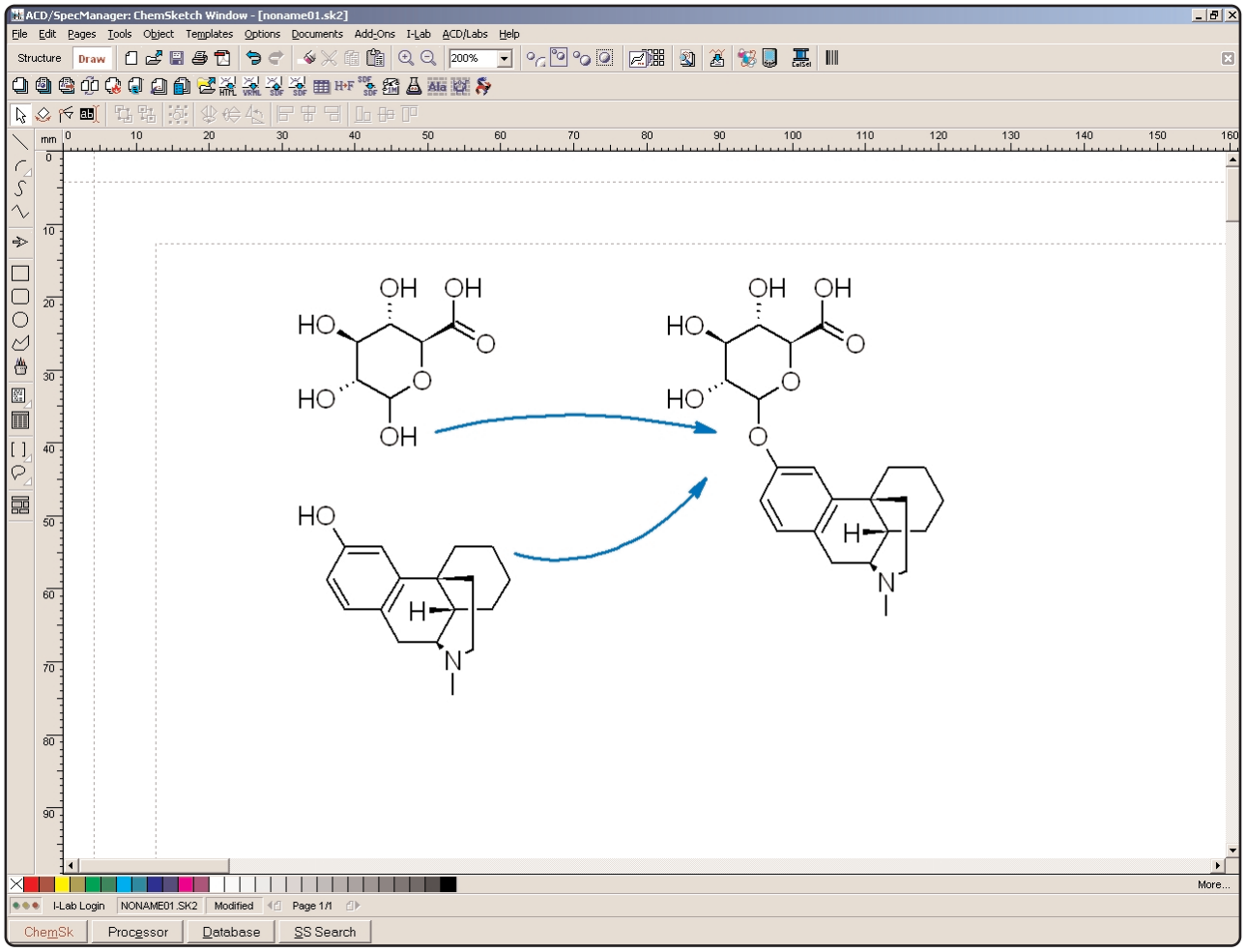

Figure 3. The ChemSketch structure drawing program. Moving the two parts of the figure together causes elimination of water from the structure, forming the dextrorphan glucuronide conjugate.

hypothetical metabolite takes only a few clicks of the mouse. The software takes care of all valence calculations and even "knows" chemistry–simply moving two structures together causes elimination of water, just as happens during a condensation reaction.

Automatic assignment of structural features to mass spectral peaks

Using a sophisticated rules-based program, a hypothetical chemical structure may be fragmented *in silico* and the resulting fragment masses compared with those from the mass spectrum of a metabolite. This MS Fragmenter (and its related module named Assignment in MS Manager) may be used to model fragmentation (positive-mode only) by EI, ESI, APCI, and FAB through multiple stages of fragmentation. It also handles multiply charged species and accurate mass fragment assignments. After a list of fragments is generated, the correlation between fragment, structure, and mass peak is shown graphically (see Figure 4). The masses of predicted fragments from several different structures may be compared quite rapidly to *m/z* values in a mass spectrum with this technique.

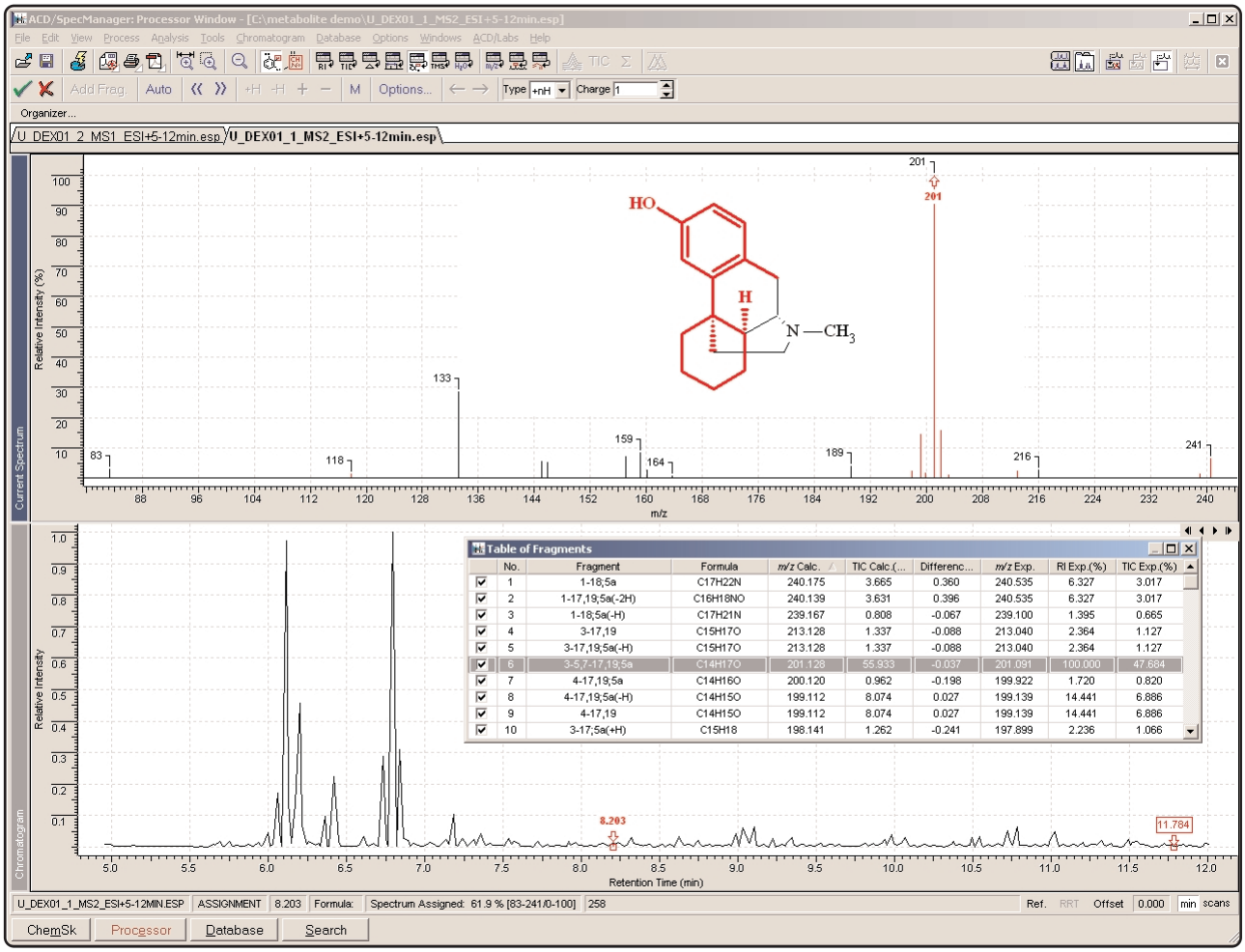

Figure 4. Assignment of structural fragments to mass spectral features. The major fragment is assigned to a substructure lacking nitrogen, consistent with positive ion mass spectrometric fragmentation rules. If desired, a user may add fragments manually to this list.

Archival of results

Mass spectra with attached structures and fragment assignments are then transferred to user databases for documentation, archival, and retrieval. The SpecDB module acts much like a searchable electronic notebook for analytical data. Each record in a database may contain multiple spectra (even from different analytical instruments), chromatograms, acquisition conditions, user data, and a structure (see Figures 5 & 6). A database can be searched using any of the stored information, even analytical information (presence of a certain mass value, for instance).

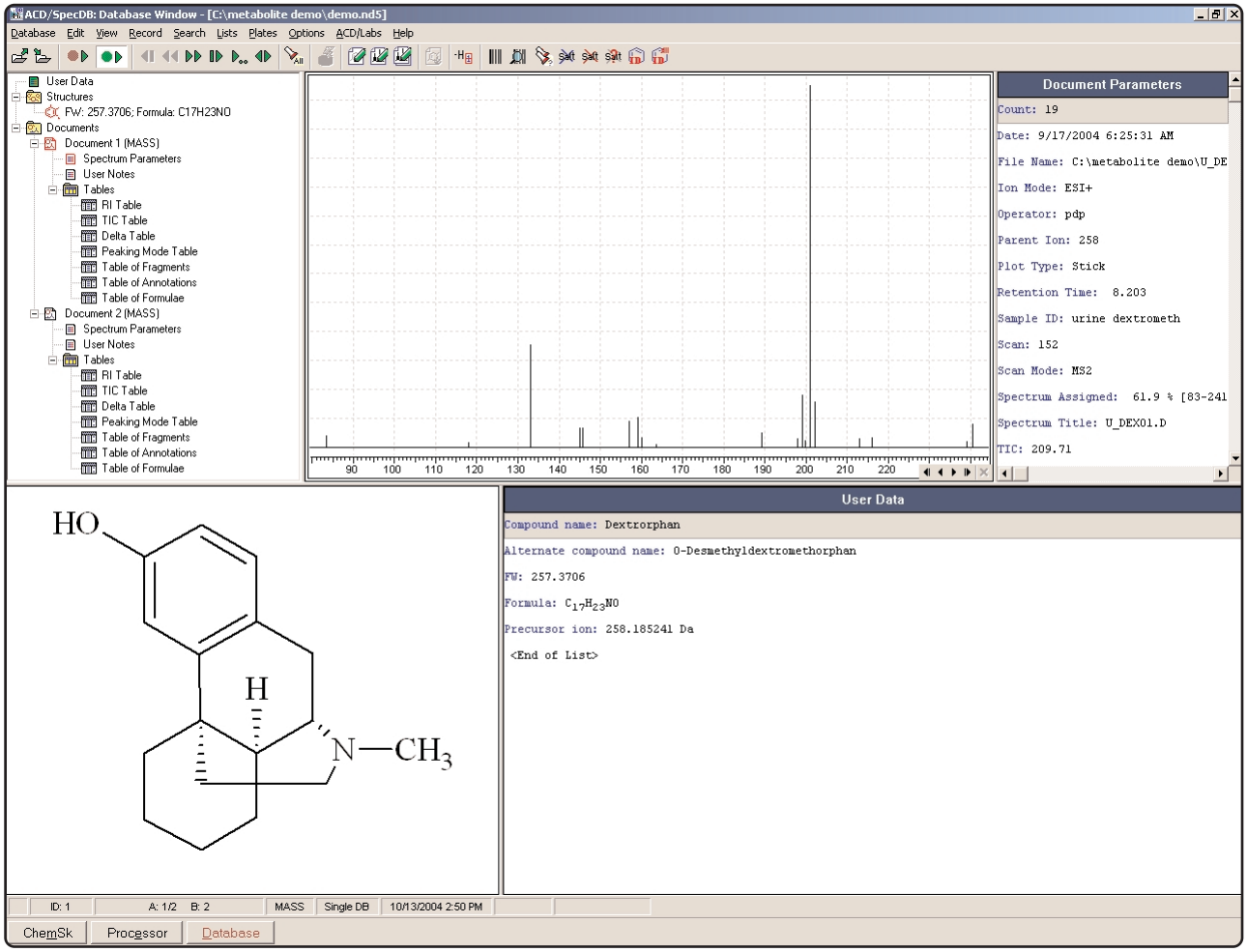

Figure 5. Database of results. Mass spectra, fragmentation assignments, user data, and chemical structures are stored for easy search and retrieval.

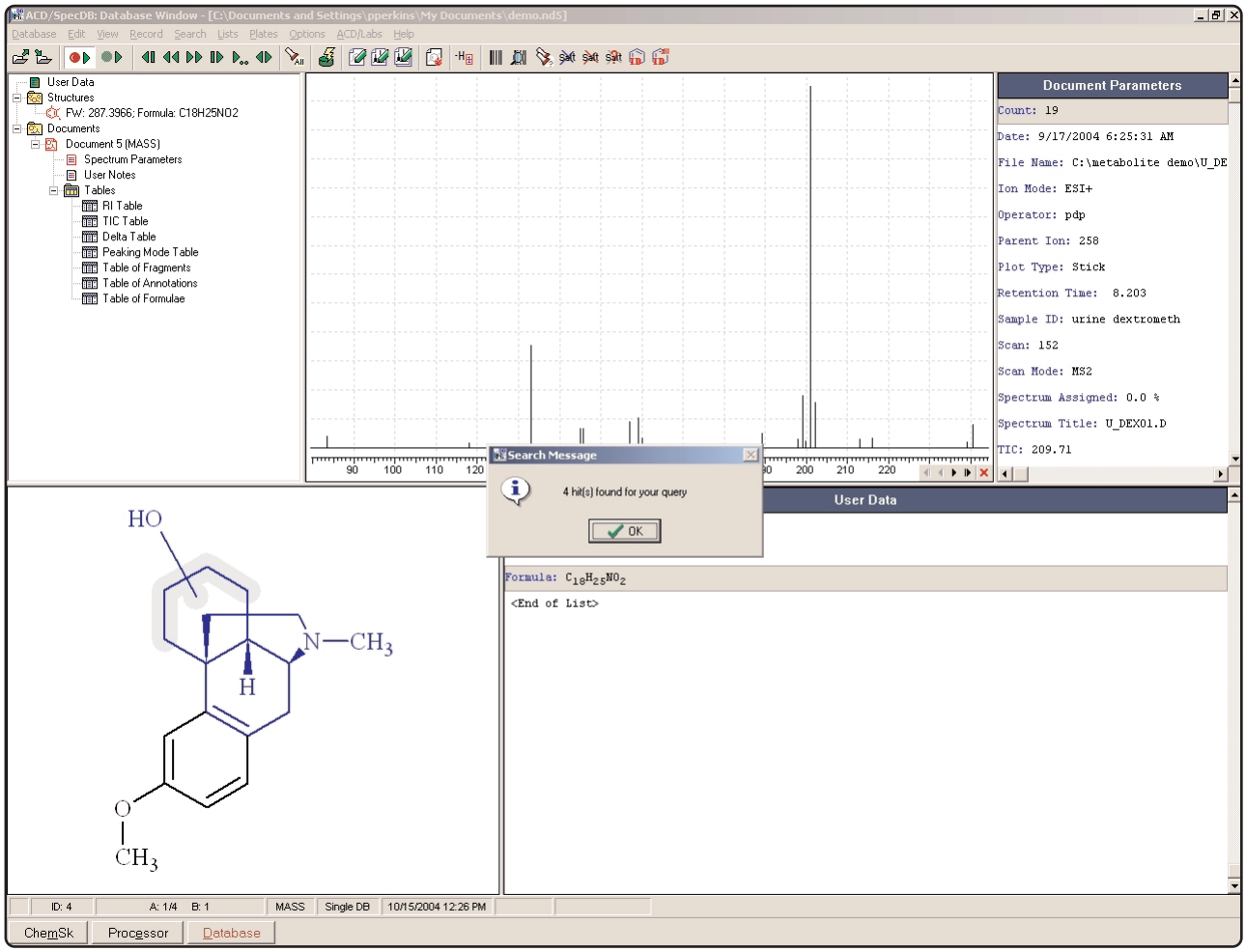

Figure 6. Substructure search results. All entries containing a given substructure may be retrieved. This includes searching for structures having ambiguous substituent locations (Markush structures).

Management of biotransformation maps and information

Once the metabolites are identified, the relationships between them and the parent drug can be illustrated as reactions using the ChemSketch module. This biotransformation map (Figure 7) is then passed to the ChemFolder module, another database program. This program links relevant information to the structure(s) or reaction(s), permitting facile management and retrieval of all information pertaining to the metabolic process(es).

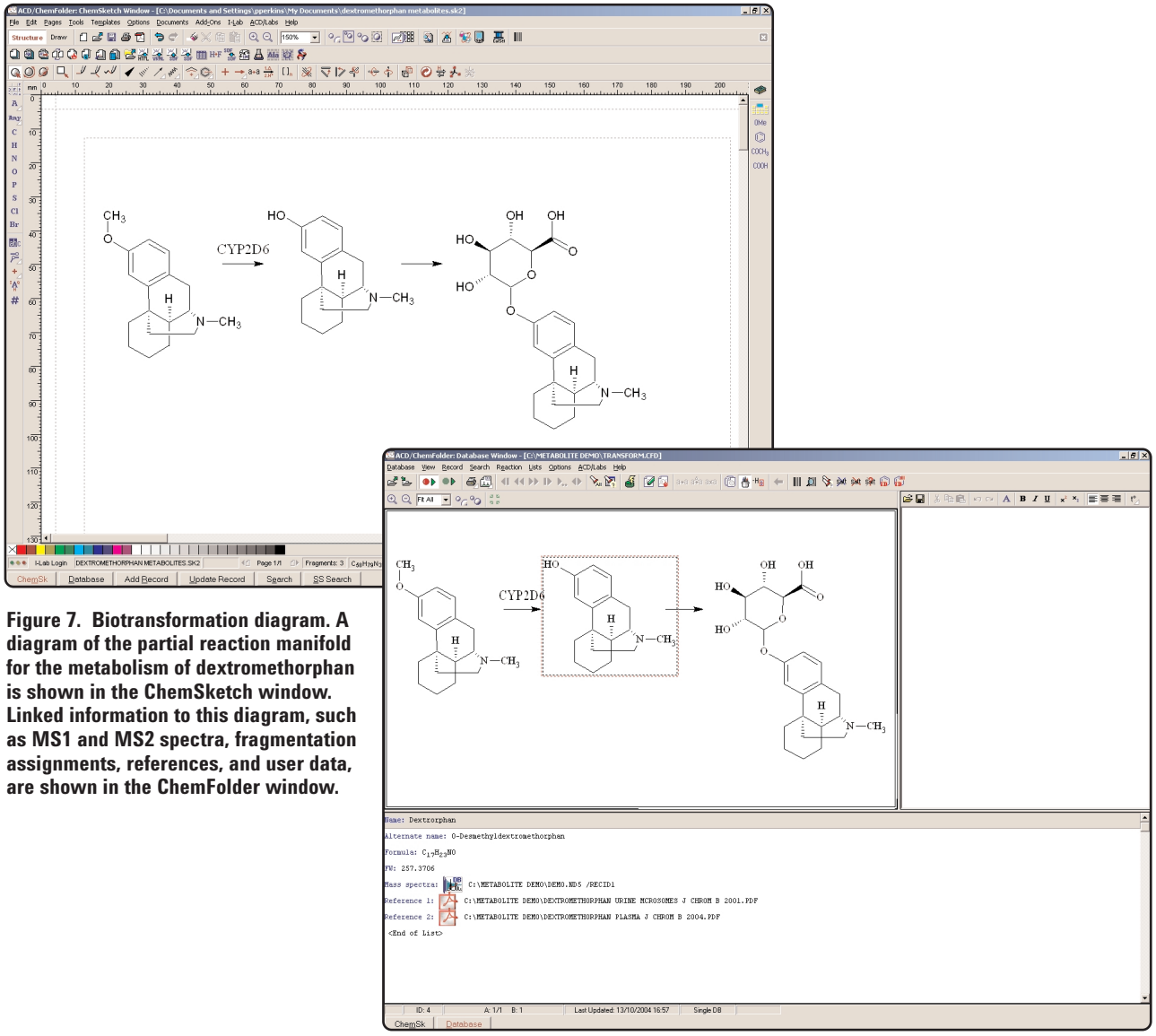

Figure 7. Biotransformation diagram. A diagram of the partial reaction manifold for the metabolism of dextromethorphan is shown in the ChemSketch window. Linked information to this diagram, such as MS1 and MS2 spectra, fragmentation assignments, references, and user data, are shown in the ChemFolder window.

Conclusions

The ACD/MS Manager software provides a powerful set of data reduction and data management tools for analyzing MS data (and data from other analytical techniques) produced from metabolism studies. These tools make it easier for less experienced scientists to interpret their own mass spectra and allow experienced mass spectrometrists to save time in their interpretations. The Agilent LC/MSD ion trap systems are designed for high sensitivity, ruggedness, and ease-of-use. This powerful combination provides the user with both high-quality LC/MS^n data and advanced data processing for drug metabolism work.

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