application

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Retention Time Locking: Creating Custom Retention Time Locked Screener Libraries

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Introduction

Retention time locking (RTL) is a powerful means for duplicating compound retention times between different HP 6890 gas chromatographs (GCs), regardless of detection method.[1–3] On any particular 6890 GC, RTL can also be useful in reproducing the original retention time of a compound following column maintenance (trimming or replacement). The highly automated implementation of RTL available with the MSD Productivity Chemstation software (version B.01.00) makes the application of RTL for GC/MS analyses very "user-friendly"[4] and it can eliminate the need to update GC/MS selected-ion-monitoring (SIM) acquisition methods and their corresponding quantitation databases.[5]

Retention time locking has allowed the creation of a specific GC/MS method and associated library (Product Number G1049A) that allows the user to "screen" samples for 567 pesticides and other prominent chemicals that are of environmental concern.[6] After acquiring GC/MS data for a sample under this method (RTLPEST.M), the RTL screening software compares the ions in a narrow window of retention times around the expected elution time of compounds in the database with those appearing in the acquired datafile. Ion abundance ratios and retention times are applied as criteria for determining "hits" and "possible hits." In one window, the Screener displays the extracted ion chromatogram of the ions for the listed compound and in another the sample ion ratios versus those expected for the compound (Figure 1). This provides very complete information for identifying and confirming the

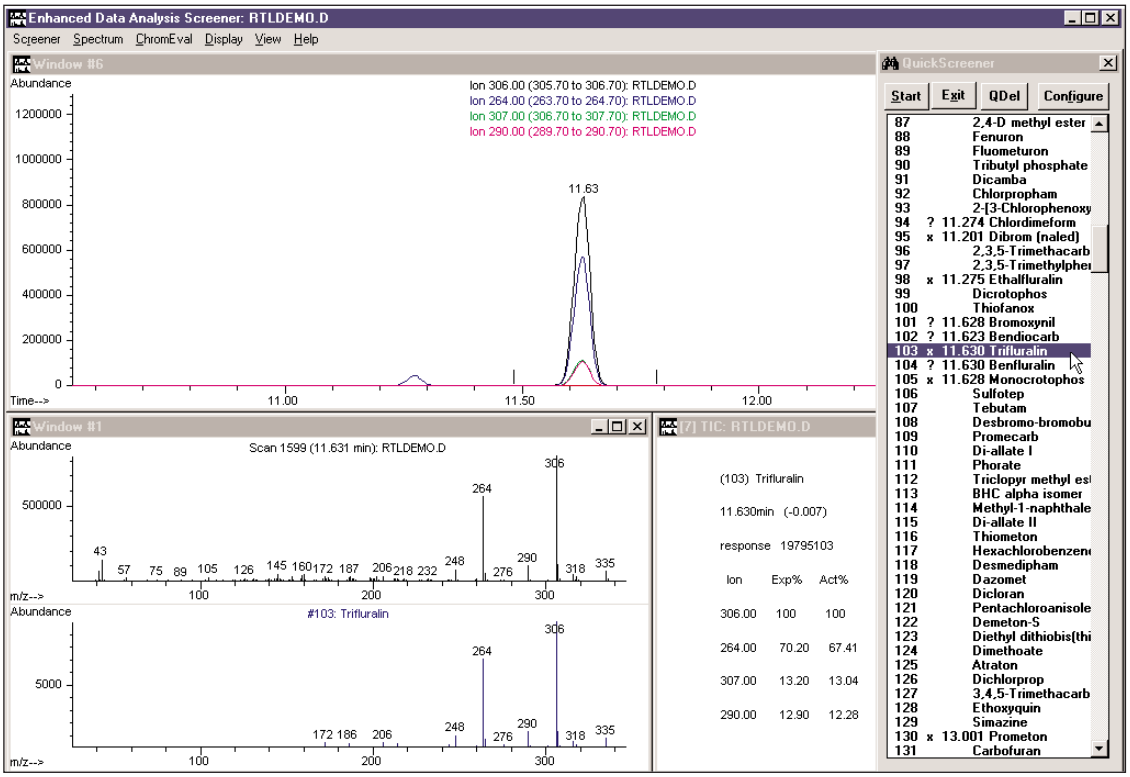

Figure 1. Screener results panels. The top window shows the extracted ions for the peak based on the compound highlighted in the Quick Screener results panel at right. The results panel indicates "hits" with an "X" and possible "hits" with a "?". The top of the lower window shows the sample peak mass spectrum and, below it, the mass spectrum of the compound being evaluated, in this example trifluralin.

presence of a compound, because both MS and RT information are applied. This has proved to be a powerful approach in expanding the number of pesticide residues that can be surveyed for in food extracts.

Recognizing that customers have their own analytical concerns and methods but that they appreciate the capability of screening for a large number of possible compounds in their samples by using locked methods, the capability for users to generate their own locked screening libraries has been implemented in the ChemStation software. The procedure for producing these libraries is described in this note. Since this procedure involves producing a mass spectral library, this note also describes how users can create their own libraries from acquired compound spectra.

It is noteworthy that these RTL libraries are *screening* libraries designed for rapid and effortless assessments of the presence of a wide variety of possible compounds that may appear in a user's samples and that they are neither meant to be nor were they designed to be quantitative. If the user is interested in quantitative compound determinations, the building of a quantitation database for a retention time locked GC/MS method is a more appropriate approach.

Software Requirements

Generating an RTL library requires MSD Productivity Chemstation software revision B.01.00 or later. The screen captures shown here were taken on version C.00.00.

Procedure

It is assumed that users have some experience with, or knowledge of, the MSD ChemStation Screener (see Reference 6) or have reviewed the online help within the software, and that they are familiar with some of the basics of working in the Enhanced Data Analysis software.

The procedure is very simple and consists of only three steps: creation of the RTL GC/MS method;[3] creation of the mass spectral library from samples acquired by the RTL GC/MS method; and conversion of the library to a screening database. It will be helpful to have the following information available before undertaking the creation of a mass spectral library: compound identity (name), molecular formula (optional) and molecular weight (optional), CAS number (optional), and the retention time for each peak (in seconds). This information can be obtained by integrating the chromatogram and running a library search on the peaks (and converting the peak time from minutes to seconds).

Creating a Retention Time Locked GC/MS Method

The necessary steps for locking a method once a suitable method has been developed are described elsewhere.[4] It is important (1) that experience with the samples be sufficient to prove that the method accords with user criteria of optimal separation in a reasonable time, and (2) that a good choice of locking compound be made. Ideally, a locking compound would be an internal standard or other compound that is known to be present in every sample and that elutes toward the middle of the chromatographic run.

Creating a Mass Spectral Library

To create a spectral library, proceed as follows: (1) Start an enhanced data analysis session, and then load the RTL method and a datafile acquired with that method; (2) Under the menu heading SPECTRUM, select the menu item EDIT LIBRARY (Figure 2). A screen will appear with the title EDIT PBM LIBRARY that offers several options, select the button CREATE LIBRARY (Figure 3). Make a new (eight-character) name for the library as *filename*.L and save it in your database directory (Figure 4).

To add a library entry, select a peak by double clicking on the TIC in Window 2. The mass spectrum should be displayed in Window 1. (The spectrum will be placed in stack register X. Type STACK on the command line, and press enter to see the contents of the stack.) You may wish to use the mass spectrum of the peak apex or the average of the peak, or background-subtract the spectrum. Under the menu heading SPECTRUM select the menu item EDIT LIBRARY. A screen will appear with the title EDIT PBM LIBRARY and offering several options; select the button ADD NEW ENTRY (Figure 5). A panel titled NEW ENTRY will appear with fields for the mass spectrum Name, Mol. Formula (optional), Mol. Weight (optional) and the Ret. Index (Figure 6). Entries *must* be made in the following fields: name, molecular weight; and retention index. If you do not know the molecular weight, enter 9999. The retention index field must contain the retention time of the peak (at the apex) in *seconds*. When the field information items have been completed, click OK and update the library (Figure 7). You must then cancel entry into the library before selecting the next spectrum. Move to the next peak, select the spectrum, and continue sequentially through the datafile until all the compounds are added. (Entries need not be made in order of retention time.) If there are other datafiles with compounds of interest, load the files and add the spectra to that library.

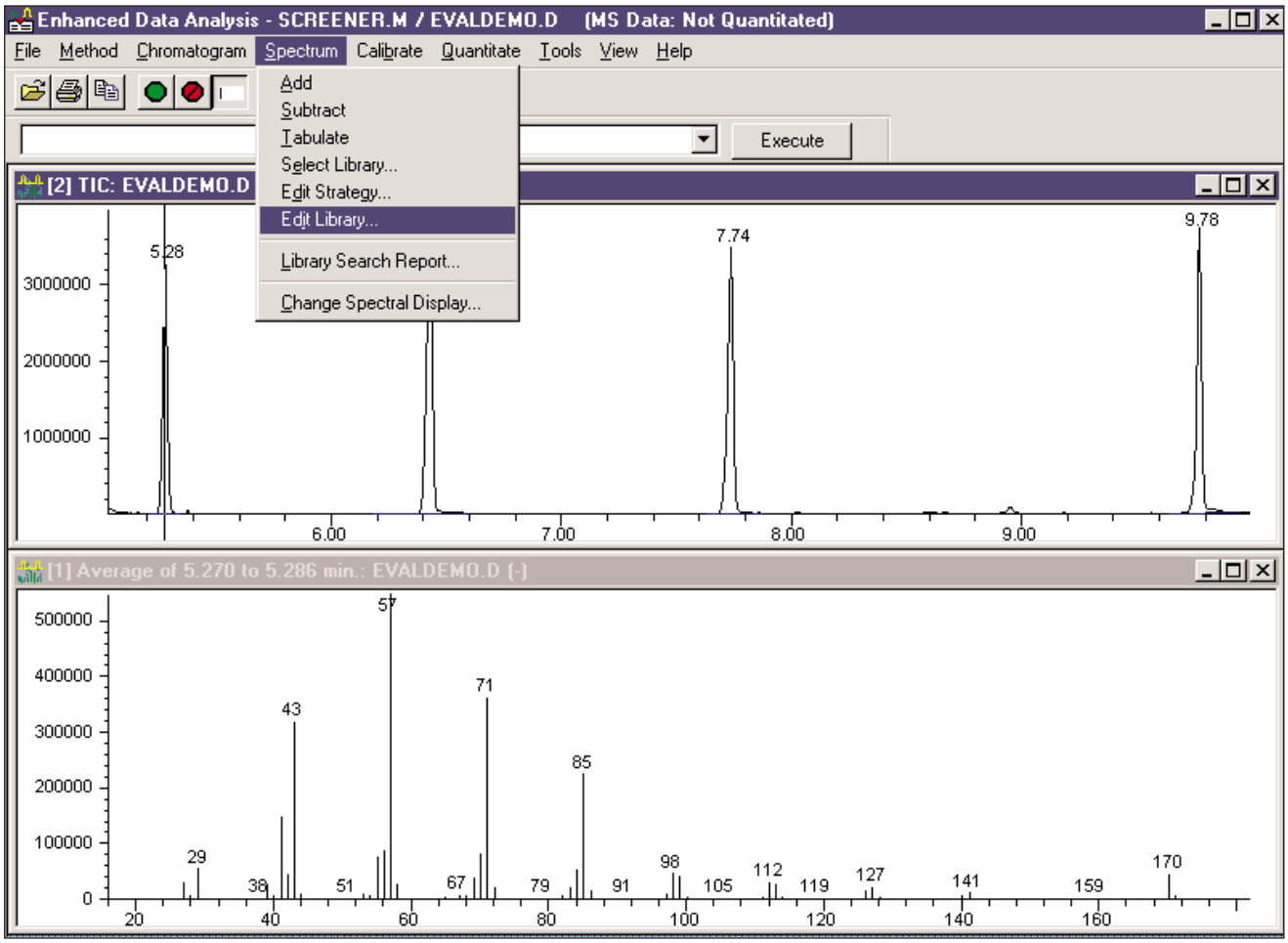

Figure 2. Selecting the EDIT LIBRARY menu item.

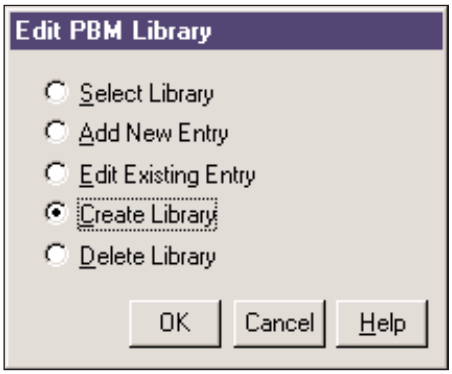

Figure 3. Selecting the CREATE LIBRARY command.

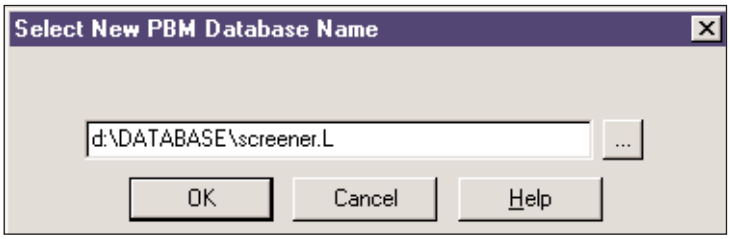

Figure 4. Naming the Screening PBM Library.

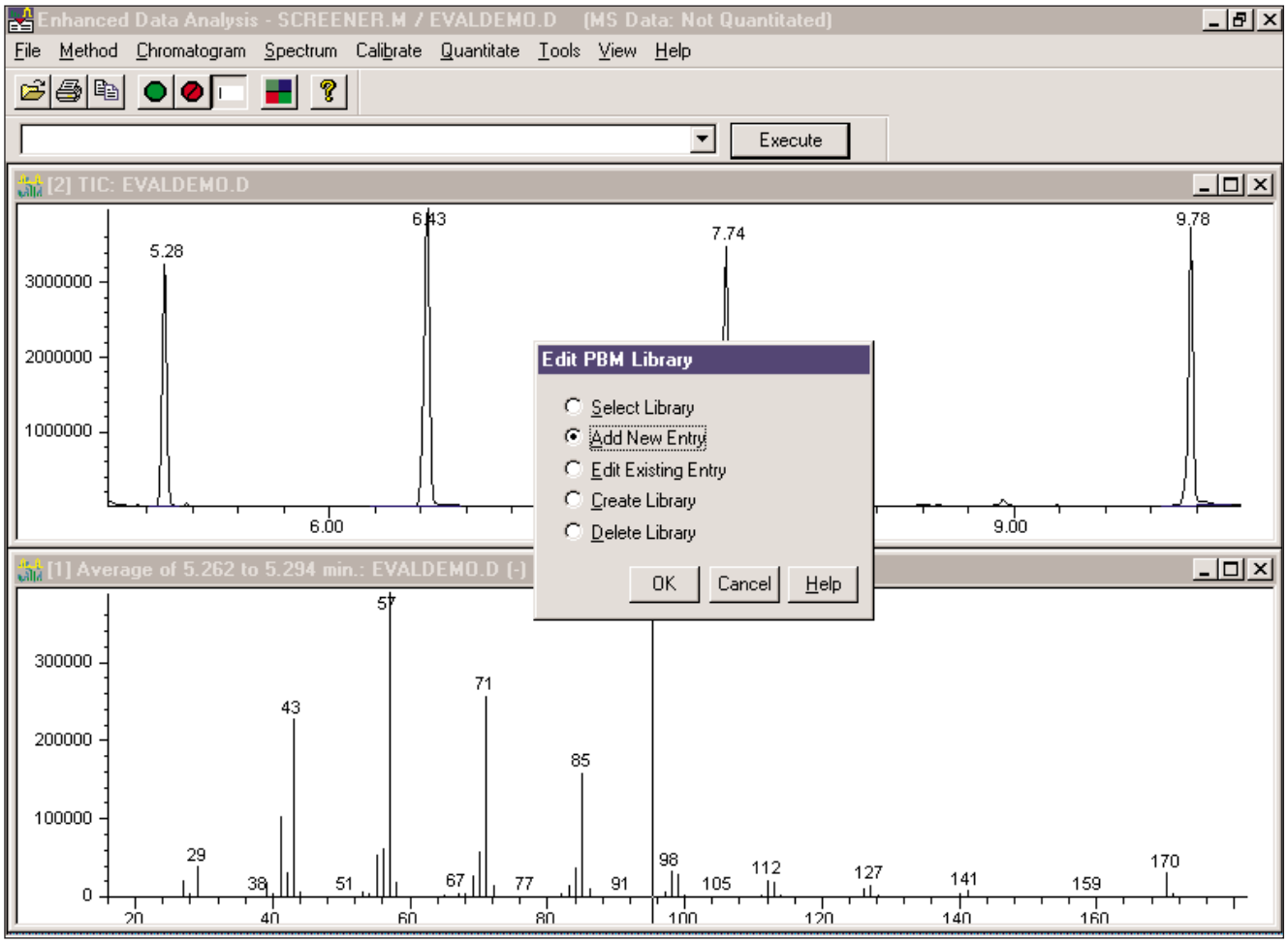

Figure 5. Adding an entry into the library. The spectrum must be visible in Window 1 and results from double-clicking the peak (equivalent to being placed in the X register of the stack).

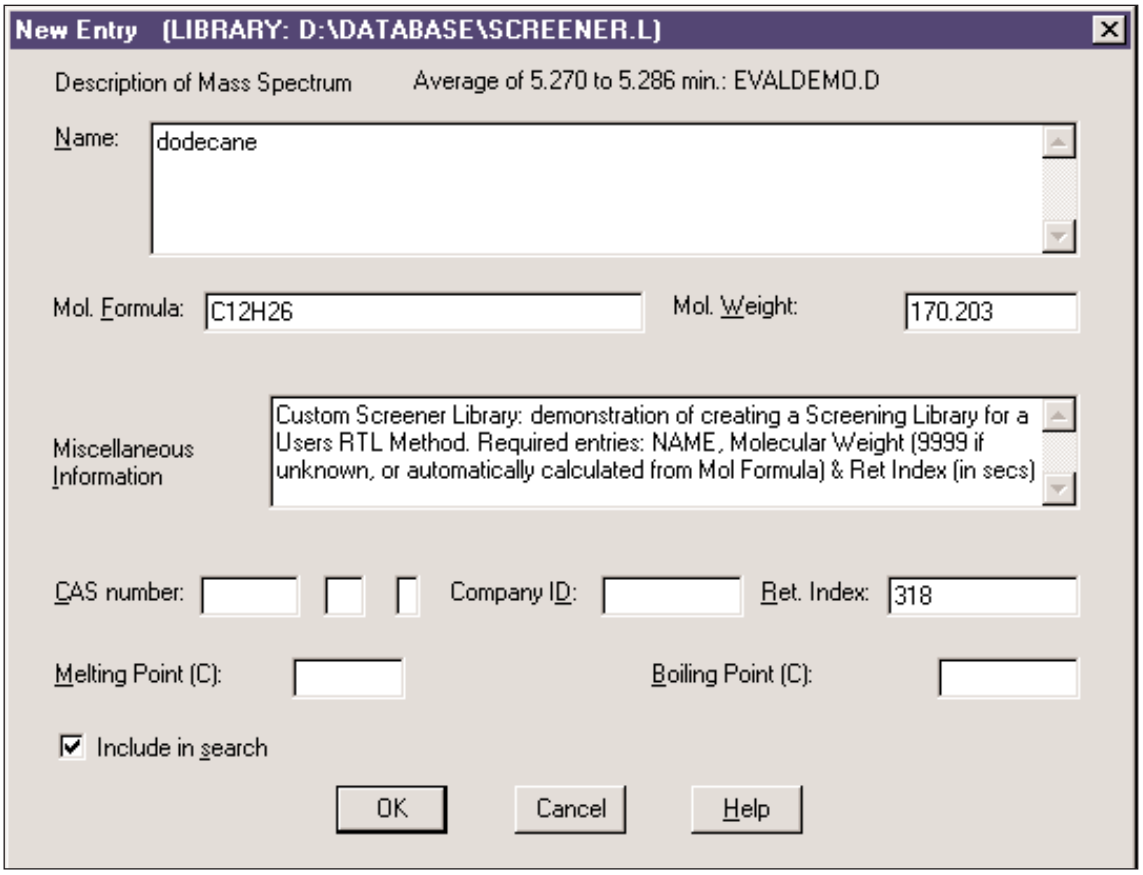

Figure 6. Library Entry panel. Required fields are the compound name; Mol. Weight, which will automatically be calculated from the Mol. Formula or, if unknown, must be entered as 9999; and Ret Index as the compound Ret. Time in seconds. This example is the first peak in the EVADEMO.D datafile.

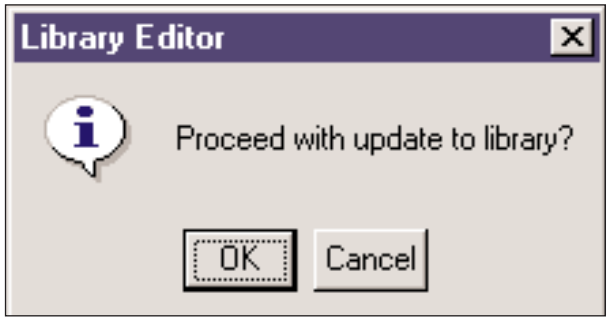

Figure 7. After an entry is made, library must be updated.

Creating a Custom Screening Library

To create a custom screening library, proceed as follows: (1) On the command line, type and then execute the command *makescreendb* (Figure 8). You will then be prompted to select the PBM library you have just created to convert to the screener database (SCD); (2) Select the YES button when prompted by the panel to "Sort *filename*.L by Retention Time?" (Figure 9). A panel will then appear prompting you to provide a name and directory in which to save your SCD file in (Figure 10).

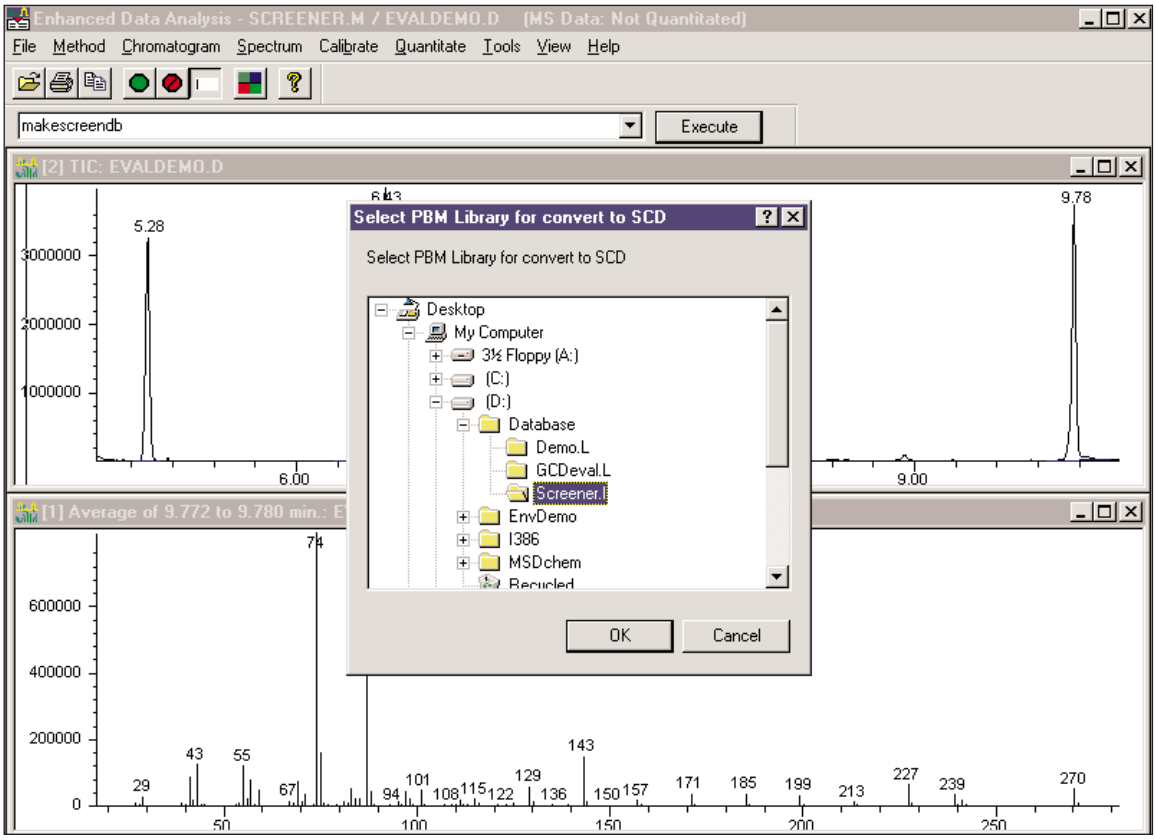

Figure 8. After executing the *makescreendb* command, you must select the newly created library that is to be converted to the screener database.

Figure 9. Select YES to sort the library.

Checking a Custom Screening Library

To check the custom screening library proceed as follows. Under the TOOLS heading, select menu item LIST SCREEN DATABASE to check the content of filename.SCD. This will display the compounds in order of retention time with their qualifying ions (Figure 11).

Also under the TOOLS heading, select the menu item SPECIFY METHOD SCREEN DATABASE and choose the appropriate filename.SCD to designate the screening library for that particular RTL method. Load a datafile that has been acquired under the locked method, then select, under the TOOLS menu, CREATE SCREEN RESULTS FOR THE CURRENT FILE. Then, under VIEW, select RESULT SCREENER. The Screener should appear with identifications for all compounds in your datafile if this was a file used to build the database (Figure 12).

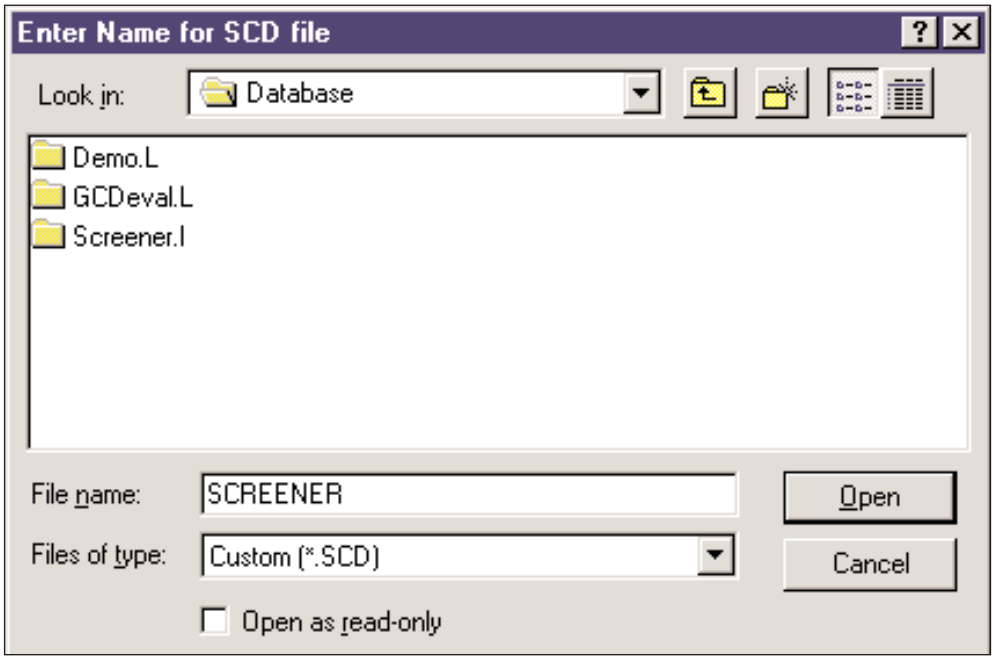

Figure 10. Select a name for the Screener Library.

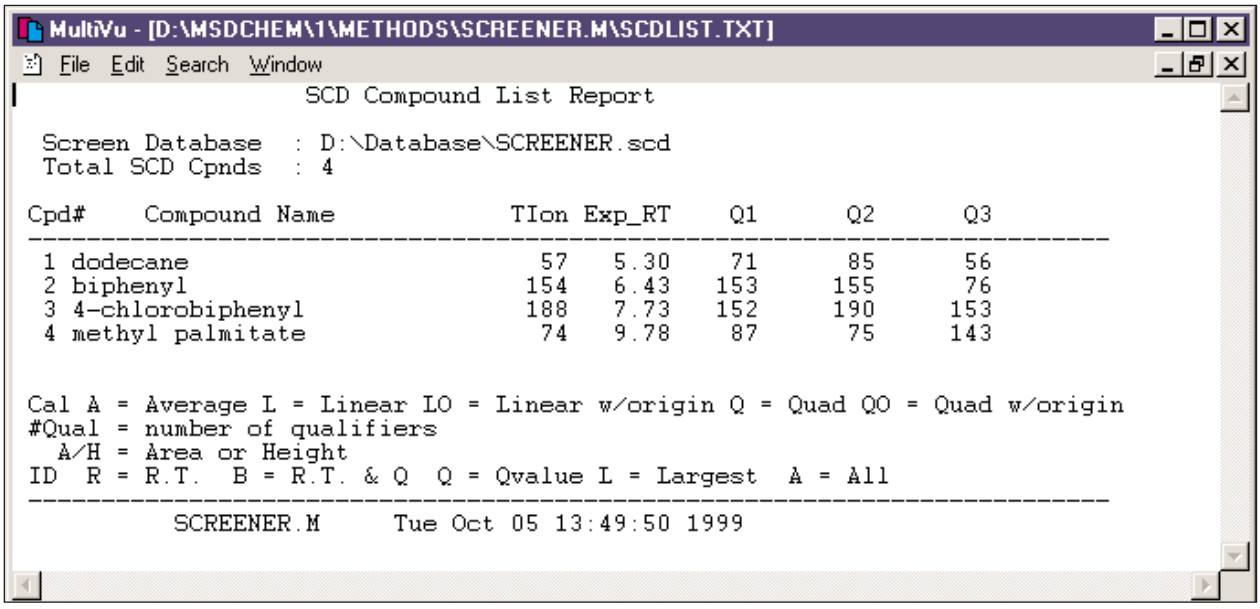

Figure 11. Output from the LIST SCREEN DATABASE menu command. This is useful for checking the Screener Database.

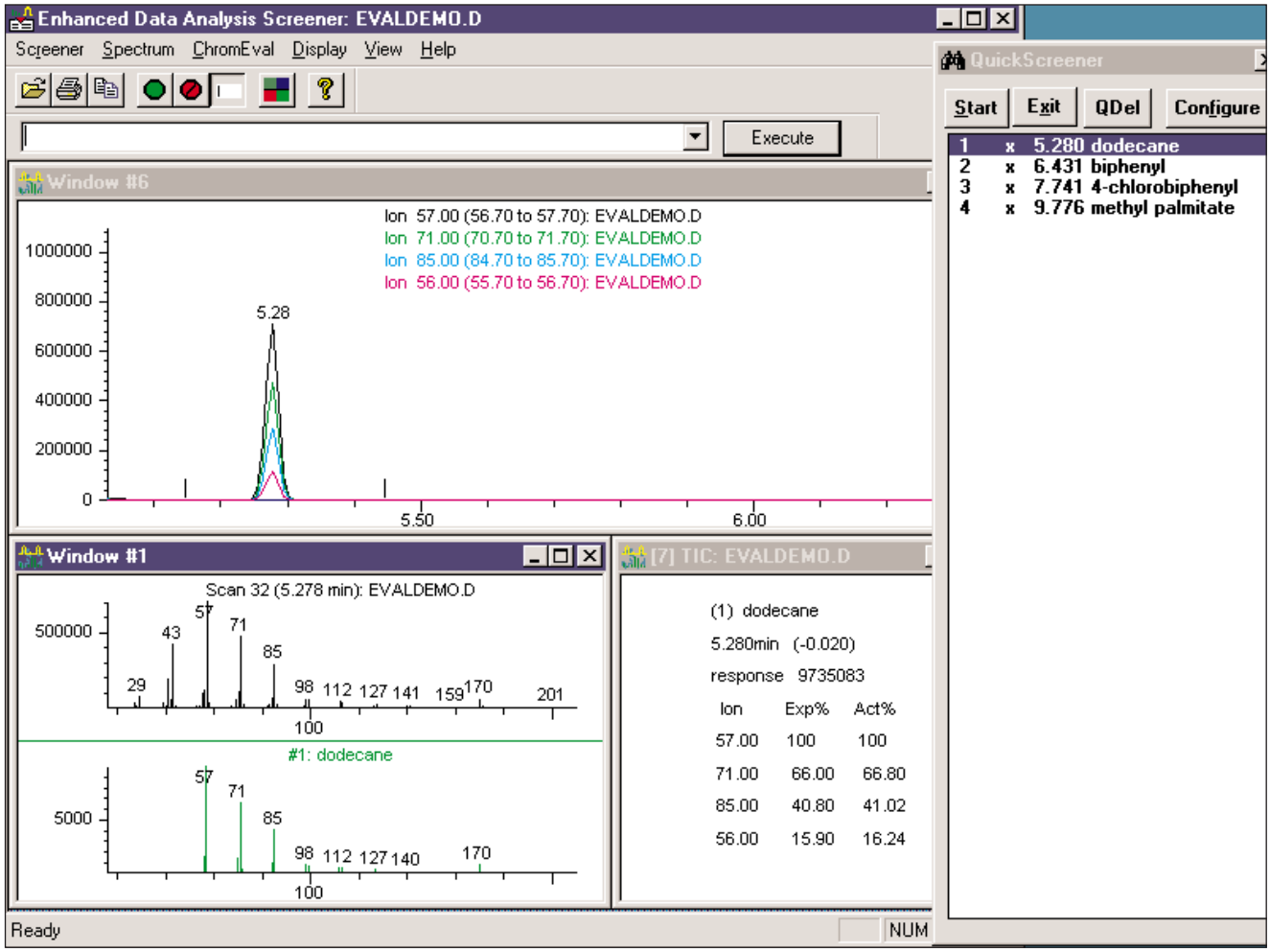

Figure 12. By selecting VIEW menu item RESULTS SCREENER, the screener will display results for the datafile loaded. Note that this is only possible if the Screener Database has been selected for that RTL method using TOOLS \ SPECIFY METHOD SCREEN DATABASE. These are the results for the EVALDEMO.D datafile.

Suggested Approach

Rather than attempt the entire procedure, use the file EVALDEMO.D provided in the DATA directory to test the procedure. This is a four component mixture (dodecane, biphenyl, 4-chlorobiphenyl, and methyl palmitate) which would allow the user to rapidly explore throughout all steps of the process in creating the library except developing the RTL acquisition method. This short screening library has to be attached to a locked method before the screener can be tested, so load the method RTLPESTF.M or RTLPESTB.M into data analysis, attach your *TEST*.SCD screening database as the screening database, and save the method as TEST.M.

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