Using the Expanded Capabilities of the LC/MSD Trap Software Version 4.2

Application Note

Frank Kuhlmann, Christine Miller, Michael Zumwalt, and Bryan Miller
Agilent Technologies

Introduction

The features of the Agilent 1100 Series LC/MSD Trap software Version 4.2 expand the capabilities of this powerful ion trap mass spectrometer control and data analysis suite. These features add to the state-of-the art capabilities that were present in the previous software version.[1] The Trap 4.2 software offers the optional Dissect function for automated peak location in LC/MS-only analyses. This algorithm locates metabolites or minor impurities in complex chromatograms. The software also includes the MASCAT program for automated concatenation of Mascot generic format (*.mgf) files, providing a means to consolidate results from multiple two-dimensional LC/MS/MS analyses into a single protein database search. The instrument control software includes automatic gain calibration, which makes it easier to achieve consistent day-to-day and instrument-to-instrument results. This technical note describes these and other powerful software features.

Dissect for Automated Location of Major and Minor Sample Components

The Dissect algorithm allows the rapid, automated identification of minor sample components such as drug metabolites. Trace-level metabolites in biological samples are difficult to detect in LC/MS total ion chromatograms (TICs) because endogenous interferences produce significant background signals. Extraction of trace-level metabolite signals from background signals often requires very time-consuming data analysis, and is complicated by overlapping elution of compounds, adduct formation and matrix interferences.

The Dissect feature automates location of these minor components in complex MS datasets. This algorithm first generates and integrates extracted ion chromatograms (EICs) for each mass, and then decides (based on symmetry, broadness, and other factors) which are valid chromatographic peaks. The program then uses fuzzy logic to eliminate noise spikes and to group the peaks into compounds. For the latter step, the algorithm considers peak width, intensity, symmetry,

signal-to-noise (S/N) and retention time to statistically determine whether two mass peaks are derived from one or two compounds. Third, the algorithm obtains clean mass spectra for each partially-coeluting compound, by calculating intensities of masses from these compounds, background subtracting, and correcting for overloaded mass intensities.

Figure 1 shows the Dissect dialog box, with its straightforward settings. The **S/N threshold** controls how many EICs are generated in the first step of the algorithm, with larger values resulting in fewer EICs. The **Cut-off intensity** establishes a threshold relative to the most abundant signal. A higher cutoff eliminates minor ions from the mass spectra and accelerates data processing. The **Maximum number of overlapping compounds** is used in the last step of the algorithm to control the separation of the mass spectra from coeluting peaks. Lower values accelerate the chromatogram processing.

Figure 2 shows Dissect applied to the LC/MSD Trap analysis of rat liver metabolites of an α-1a antagonist drug. The Dissect function located 26 metabolite peaks in less than a minute. Results compared favorably with those obtained by an

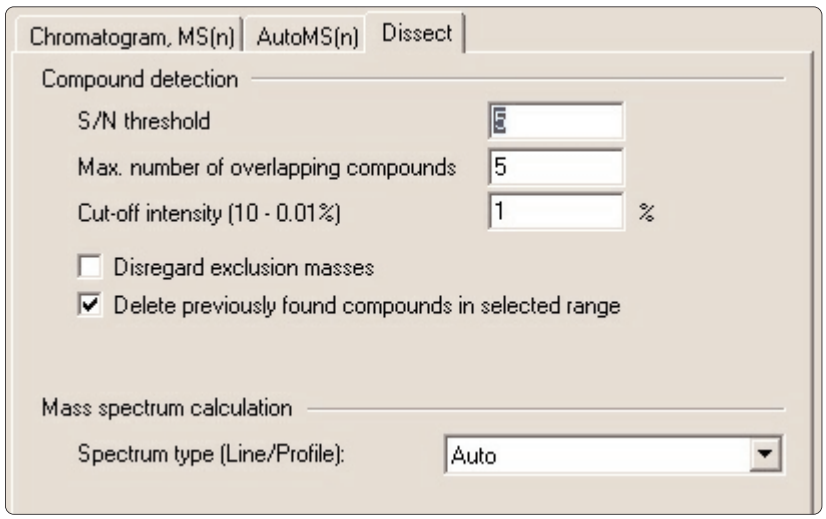


Figure 1. Dissect settings

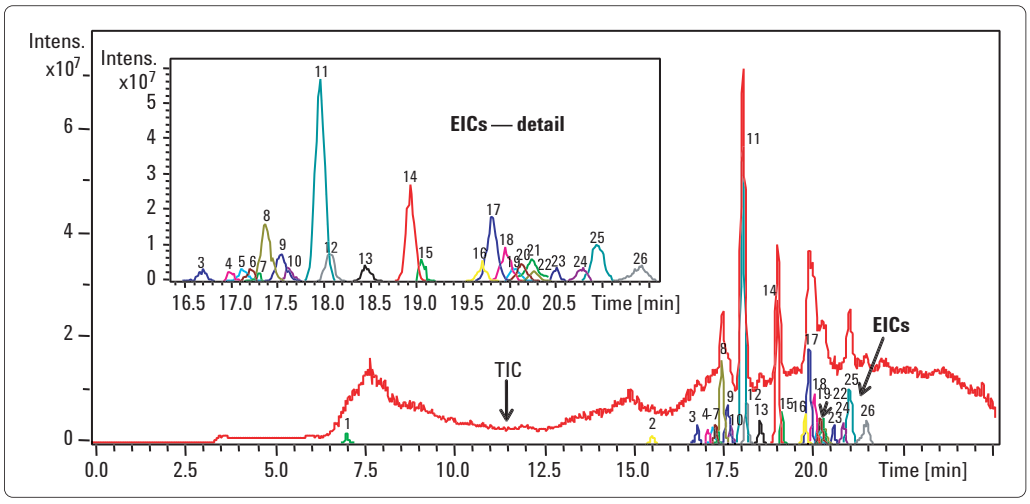


Figure 2. Dissect results—26 metabolite peaks located automatically in less than a minute

hour's worth of tedious manual identification. Moreover, even though there were many coeluting compounds, the mass spectra reconstructed by the algorithm were relatively pure.

MASCAT

Two-dimensional LC/MS/MS is a powerful tool for proteomics analyses. Samples are fractionated automatically, reducing the number of coeluting peptides, increasing the number of unique MS/MS analyses, and increasing the amount of sequence information. This type of analysis presents a challenge, however, in organizing and evaluating results from multiple sample fractions. Without advanced tools, the user must perform a separate database search for each fraction and manually consolidate the results. Even then, some proteins may not be found when results are processed this way.

The MASCAT program, a standard feature of the LC/MSD Trap software Version 4.2, greatly reduces the burden of data interpretation and organization for this type of analysis. MASCAT concatenates multiple *.mgf files for Mascot database search, enabling consolidated search of spectra from two-dimensional LC/MS/MS analyses. Figure 3 shows the MASCAT dialog box. In the upper right, an entire directory of data files has been selected by clicking a single button. In the middle left, the number of required Mascot

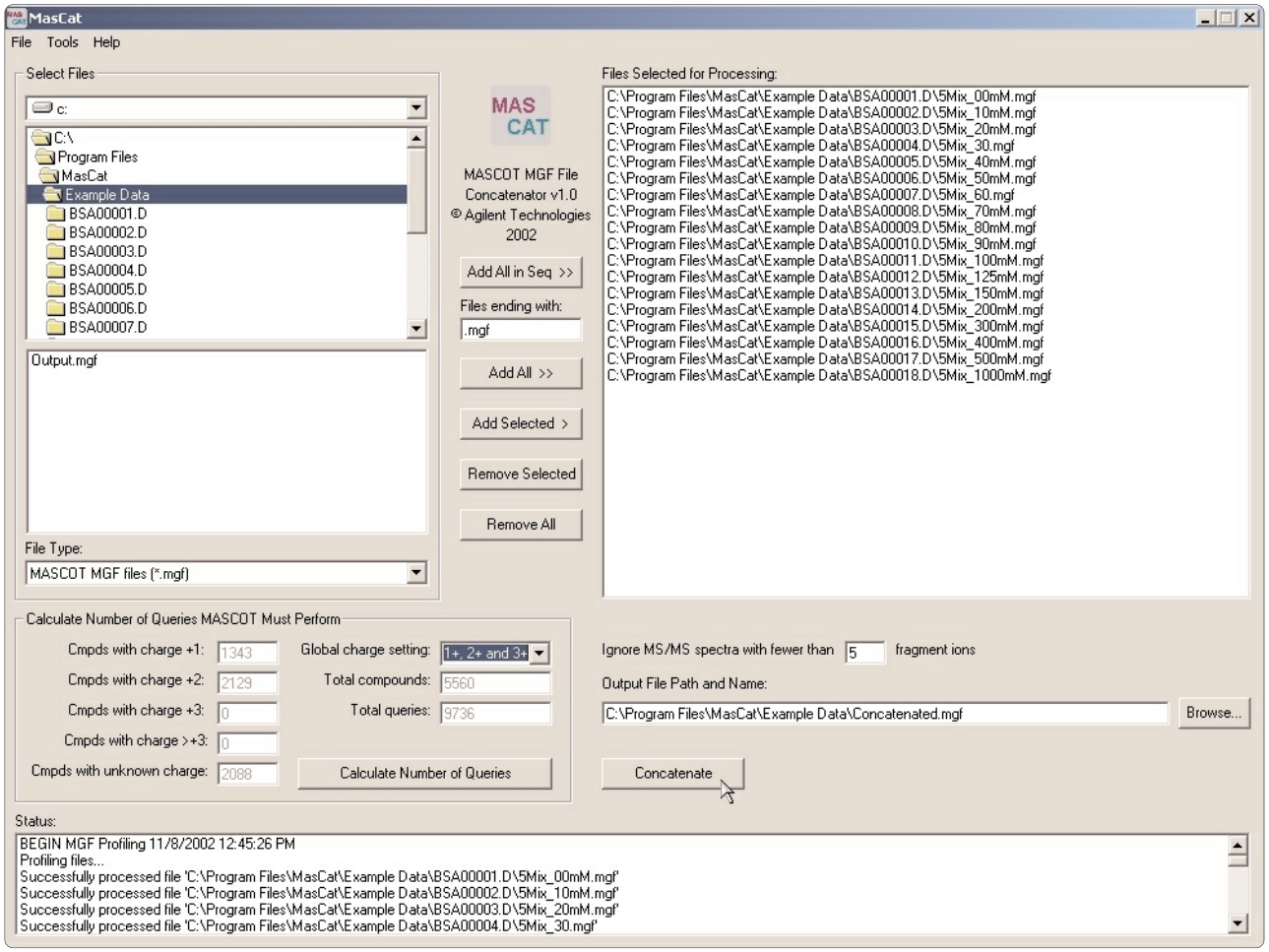
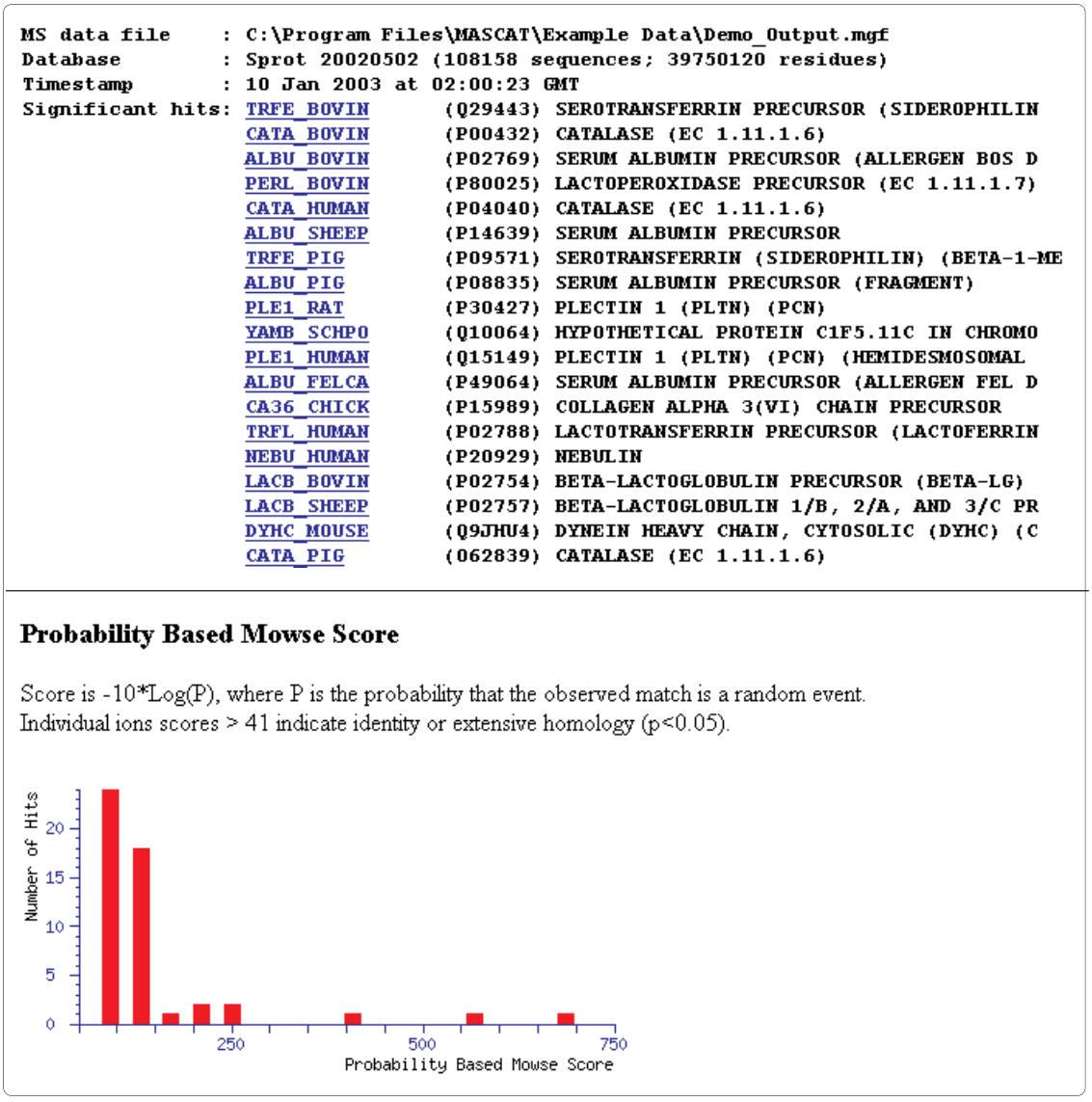


Figure 3. MASCAT dialog box for concatenating Mascot generic files from multiple 2D LC/MS/MS analyses

queries has been calculated, based on the number of spectra to be searched and the number of permutations required to consider all possible charge states. By having prior knowledge of the number of queries, the scientist immediately knows if the total number of searches will become excessive in terms of search times or memory requirements. If so, the user can regenerate the individual Mascot generic files using a higher threshold for compound finding or use the "Ignore MS/MS spectra with fewer than . . . fragment ions" parameter to eliminate low-quality spectra from the .mgf file. Figure 4 shows Mascot search results from a concatenated *.mgf file.



```
MS data file     : C:\Program Files\MASCAT\Example Data\Demo_Output.mgf
Database         : Sprot 20020502 (108158 sequences; 39750120 residues)
Timestamp        : 10 Jan 2003 at 02:00:23 GMT
Significant hits: TRFE_BOVIN      (Q29443) SEROTRANSFERRIN PRECURSOR (SIDEROPHILIN
                  CATA_BOVIN      (P00432) CATALASE (EC 1.11.1.6)
                  ALBU_BOVIN      (P02769) SERUM ALBUMIN PRECURSOR (ALLERGEN BOS D
                  PERL_BOVIN      (P80025) LACTOPEROXIDASE PRECURSOR (EC 1.11.1.7)
                  CATA_HUMAN      (P04040) CATALASE (EC 1.11.1.6)
                  ALBU_SHEEP      (P14639) SERUM ALBUMIN PRECURSOR
                  TRFE_PIG        (P09571) SEROTRANSFERRIN (SIDEROPHILIN) (BETA-1-ME
                  ALBU_PIG        (P08835) SERUM ALBUMIN PRECURSOR (FRAGMENT)
                  PLE1_RAT        (P30427) PLECTIN 1 (PLTN) (PCN)
                  YAMB_SCHPO      (Q10064) HYPOTHETICAL PROTEIN C1F5.11C IN CHROMO
                  PLE1_HUMAN      (Q15149) PLECTIN 1 (PLTN) (PCN) (HEMIDESMOSOMAL
                  ALBU_FELCA      (P49064) SERUM ALBUMIN PRECURSOR (ALLERGEN FEL D
                  CA36_CHICK      (P15989) COLLAGEN ALPHA 3(VI) CHAIN PRECURSOR
                  TRFL_HUMAN      (P02788) LACTOTRANSFERRIN PRECURSOR (LACTOFERRIN
                  NEBU_HUMAN      (P20929) NEBULIN
                  LACB_BOVIN      (P02754) BETA-LACTOGLOBULIN PRECURSOR (BETA-LG)
                  LACB_SHEEP      (P02757) BETA-LACTOGLOBULIN 1/B, 2/A, AND 3/C PR
                  DYHC_MOUSE      (Q9JHU4) DYNEIN HEAVY CHAIN, CYTOSOLIC (DYHC) (C
                  CATA_PIG        (062839) CATALASE (EC 1.11.1.6)
```

Probability Based Mowse Score

Score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 41 indicate identity or extensive homology (p<0.05).

Figure 4. Partial Mascot search results from a concatenated *.mgf file from eighteen 2D LC/MS/MS analyses. Shown are some of the fifty top hits, which were all above the significance level.

Automated Electron Multiplier Gain Adjustment

The Trap 4.2 software has an algorithm for automatically calibrating the electron multiplier gain. While setting the gain in effect adjusts the electron multiplier voltage (EMV), there are advantages to calibrating and setting gain rather than merely setting the EMV. Once the gain has been calibrated so the relationship between EMV and signal response is known, the gain setting can be used to maintain constant signal response from day to day and from instrument to instrument. Also, because the relationship between gain and signal abundance is linear (whereas EMV and signal abundance is not), it is easier to adjust methods using a gain setting than using an EMV setting. These features of gain calibration make it easier to develop methods and to transfer methods from one instrument to another. Figure 5 shows the dialog box used for calibrating gain.

Additional New Features

The LC/MSD Trap software Version 4.2 has other features to increase productivity and to address a wider range of applications.

ChemStation and LC/MSD Trap control enhancements

This software includes the Agilent ChemStation software revision A.09.03, with support for:

- The 1100 Series nanoflow LC pump, designed for trouble-free proteomics applications
- The 1100 Series valves, developed for more flexible solvent and column selection, increased throughput via alternating column regeneration, higher productivity with automated sample enrichment and sample cleanup, and better separation performance with multidimensional chromatography
- The well-plate autosampler capacity extension module, designed for high-throughput and walk-up capabilities

Other LC/MSD Trap control enhancements include reduced data file sizes attributable to an on-the-fly loss-free compression, capability for MS method modification by loading parameters from a CSV file, and improved atmospheric pressure photoionization (APPI) ion source support.

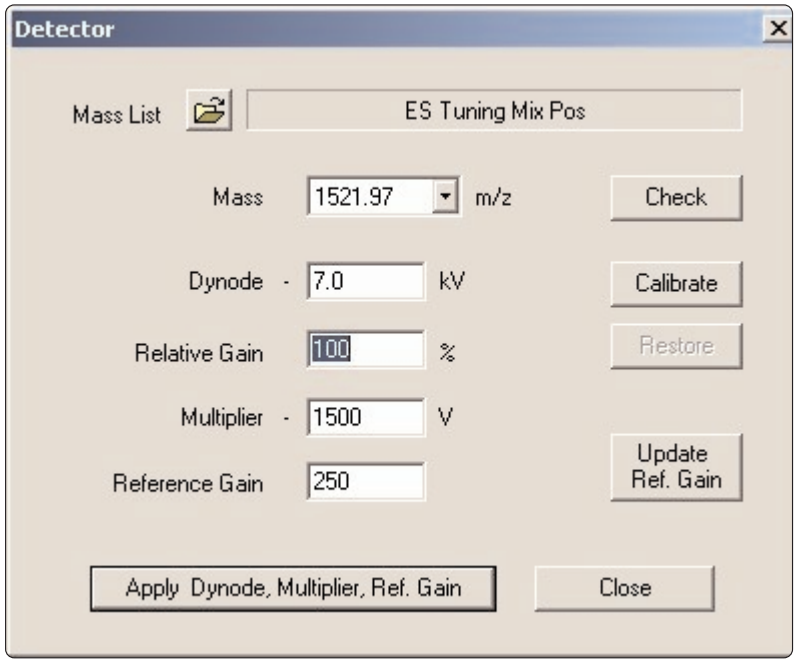


Figure 5. Dialog box to check and calibrate detector gain

Data analysis and automation enhancements

The LC/MSD Trap software Version 4.2 makes it easier to perform MS background spectrum subtraction from each compound mass spectrum extracted by the Find Compounds algorithm. Users can have the software subtract a user-selected single or averaged background spectrum, or a background spectrum determined automatically by averaging the start and end spectra of the compound peak.

Previous versions of this software have allowed data acquisition using time segments, where each segment can consist of a completely different set of ion trap parameters. Now these time segments can be displayed in DataAnalysis using vertical dashed lines in the chromatogram window. This makes it easy to visualize where acquisition conditions changed during the chromatographic analysis.

The software includes enhancements for analyzing multiply-charged compounds, including proteins and peptides. Line spectra can now be deconvoluted, and global charge-state assignments have been adapted for MASCOT 1.8, allowing the flexibility of setting a global charge limitation which includes singly, doubly, and triply charged ions.

The DataAnalysis software includes other enhancements, such as:

- Extraction of UV chromatograms from DAD spectral data, interactively or via automation commands
- Display of acquisition parameters for averaged mass spectra
- Extensions to the automation command set
- Progress bar and cancel button for lengthy commands
- Modeless DataAnalysis method parameter dialog box, which allows users to test settings without closing the dialog box.

Additional enhancements

QuantAnalysis enhancements include an Individual Analysis Report, as well as the ability to load and process data from two or more sequences. ReportDesigner enhancements include an LC acquisition parameter report component, enhanced report element grouping capabilities, and a new manual and significantly expanded online help.

Conclusions

The LC/MSD Trap software Version 4.2 builds upon the existing features of the LC/MSD Trap software and adds capabilities to enhance productivity and ease-of-use.

Acknowledgment

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References

1. "Agilent 1100 Series LC/MSD Trap Software Demonstrations," **http://www.chem.agilent.com/Scripts/Generic.ASP?lPage=6690**.

Authors

Frank Kuhlmann, Christine Miller, Michael Zumwalt, and ***Bryan Miller*** are scientists at Agilent Technologies in Santa Clara, California U.S.A.

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