Using accurate mass LC/MS in a walk-up environment

Application Note

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Abstract

Accurate mass measurements can be used to both confirm the presence of a compound and help identify unknowns. Obtaining accurate measurements using mass spectrometry has typically required highly skilled operators doing interactive instrument operation and data manipulation. This Application Note describes how using the Agilent LC/MSD TOF and new software for walk-up operation, chemists can get the accurate mass information they need easily and without much training.

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Introduction

Instruments providing accurate mass have been used in the development of pharmaceuticals both for confirming the identity of synthesized compounds and as a powerful tool in the identification of unknowns. However, obtaining the needed mass accuracy has required the samples to be run by highly skilled mass spectrometrists. In today's pharmaceutical development laboratories there is a desire for samples to be run by the chemists and not by dedicated operators. The Agilent LC/MSD TOF is capable of providing accurate mass routinely. By having a calibrant delivery system to automatically dispense calibrant and a solution for doing automated internal reference mass correction, any operator skilled enough to run a single quadrupole instrument can obtain accurate mass spectra.

Submitting samples

To make the operation even simpler, the Agilent Easy-Access software used on the LC/MSD Quadrupole was adapted for use on the LC/MSD TOF. To the sample submitter, the user interface is virtually indistinguishable from the quadrupole version. The submitter merely logs in some sample information, selects from some methods previously set up on the system by a system administrator and if empirical formula confirmation is desired, the target formula is input. The submitter is prompted where to place the samples in the autosampler and the system indicates when they will be completed. The samples are logged into a queue and when analyzed, a report is printed out or e-mailed to the submitter. The user interface is shown in figure 1. Clicking on "Submit New Sample" brings up a series of three logging screens. The first screen is shown in figure 2. The user identifies themselves and some basic information about the sample batch. Note that the labels such as

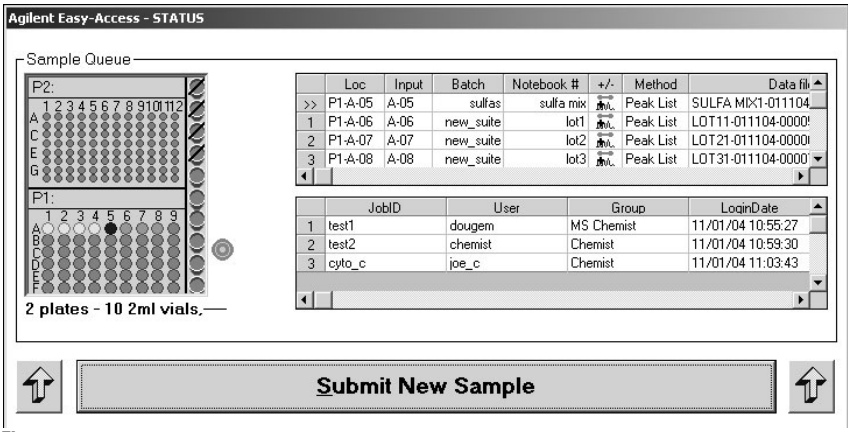

Figure 1
The Easy-Access user interface.

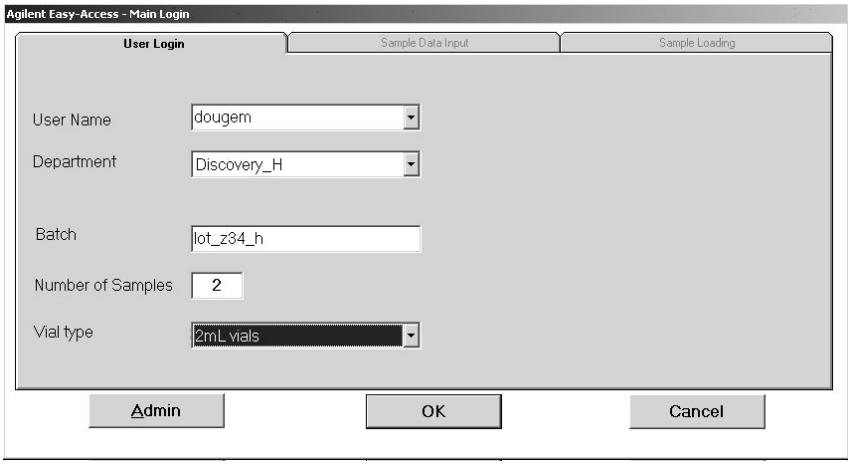

Figure 2
User Login screen for Easy-Access.

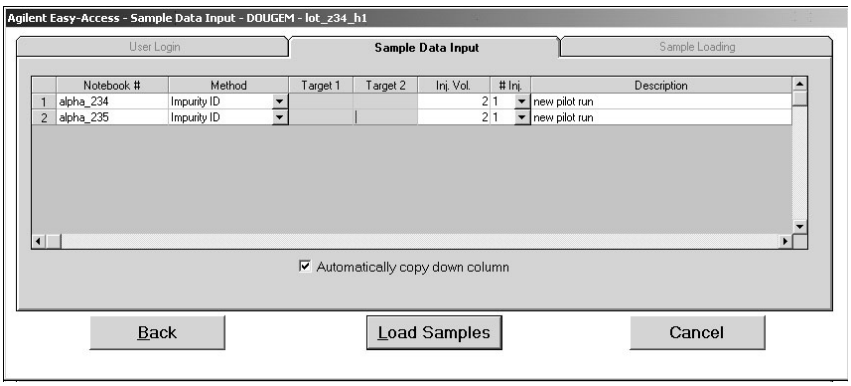

Figure 3
Sample Data Input screen for Easy-Access. The fields available depend on user capabilities.

"Department" can be renamed to whatever is the common terminology in each laboratory. Figure 3 shows the second panel where the user inputs the sample specific information. For certain reports, formulas, molecular weights or protein sequences are entered here. If the user has the capability, they can specify injection volume. Figure 4 shows the final panel where the user is prompted where to place the samples and notified of an approximate completion time. If a specified time has passed since the last sample was run, the system will automatically introduce the calibrant mixture and recalculate the time-to-mass coefficients before running these next samples.

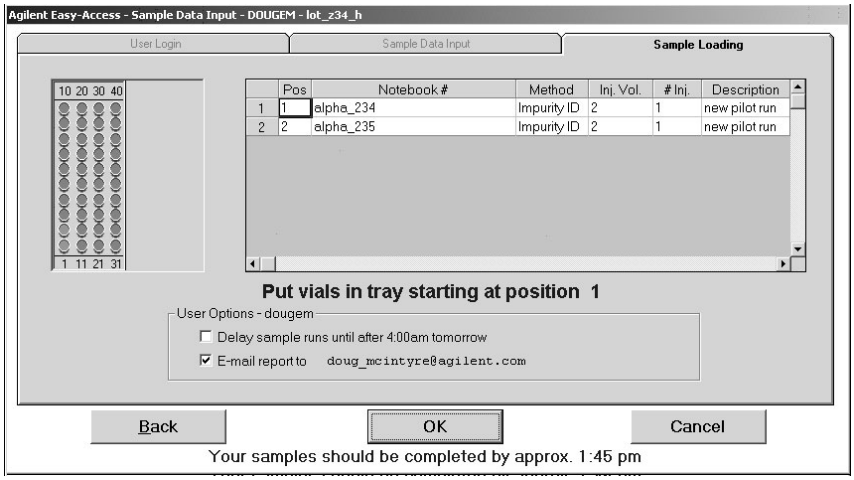

Figure 4
Final login screen indicating where samples should go and completion time.

Obtaining results

The user can either return later to receive printed reports or receive them by e-mail. A variety of automated reports can be produced by the system for either confirming the synthesis of lead compounds or aid in identifying unknowns. The automated reports include:

- Empirical Formula Confirmation (EFC) – The user inputs an empirical formula and the report indicates whether the compound is present with the calculated mass error for each adduct ion specified in the method.
- Mass List – A mass spectrum is printed out for each peak in the chromatogram and the 'n' most abundant ions are tabulated
- Empirical Formula Generation (EFG) – An extension of the mass list report where each of the ions has the possible empirical formulas calculated. The results are printed in either order of increasing mass error or isotope ratio fit.

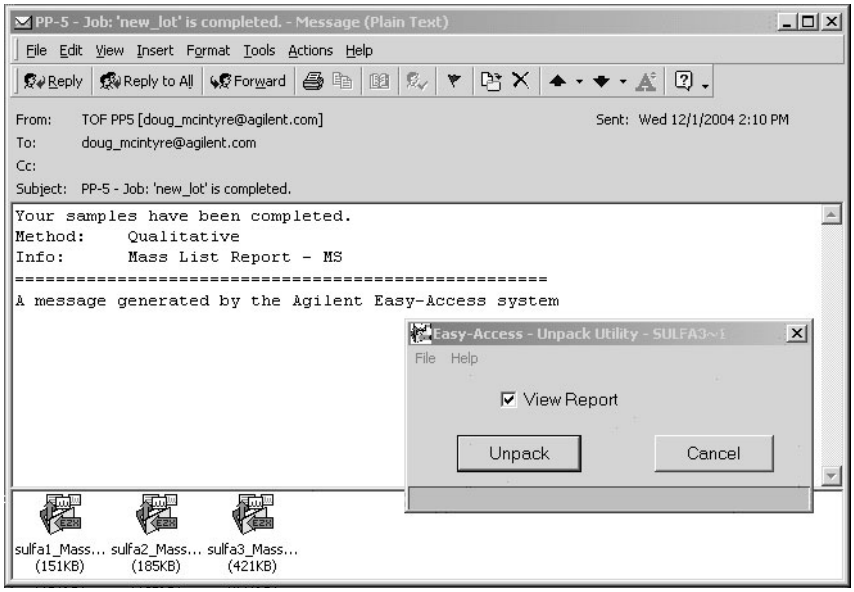

Figure 5
E-mail notification of results with attached reports.

- Confirmation Screening – An extension of the mass list report where each ion is compared against a database of compounds that contains their formulas and retention times. The results are sorted by either mass error or retention time fit.
- Confirmation Screening (reverse) – An extension of the EFC report where a database of compounds that contains their formulas and retention times is used. An extracted ion chromatogram is produced for each compound and then the spectrum is analyzed to see if it is within the minimum mass error of the target. The results are identical to the EFC report.
- Protein ID – The user inputs a molecular weight or protein

sequence and the report indicates whether a mass spectrum can be deconvoluted to produce that molecular weight. Additionally, the other major proteins are identified and their mass differences from the target protein are reported.

Figure 5 shows an example of how the user would receive an e-mailed report. Double clicking on the attachment launches an unpack utility and the report appears in Internet Explorer. The time-of-flight software is not required on the user's desktop PC.

System status

There are several ways that the system status is indicated. The main user interface indicates the samples in the queue and the samples completed as well as the time before a newly submitted sample would be run. A status bar can be optionally displayed on the top of the PC screen indicating what is presently going on. For example, if an automatic calibration is being done prior to running a sample, "calibrating" will appear on the status bar. In addition, a status box viewable over the building network indicates what sample is currently running, the queue length and other information. This is a customizable HTML file that can display the status of all instruments in the laboratory running Easy-Access, whether they are quadrupole or TOF systems. Figure 6 shows the standard version of this screen.

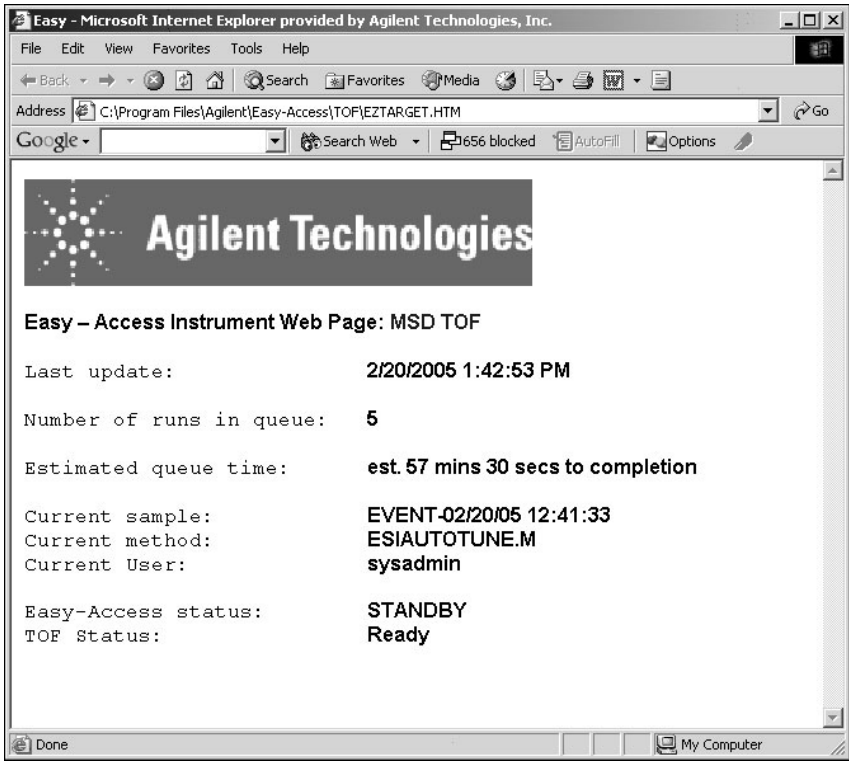

Figure 6
Web page indicating the current status of the Easy-Access system.

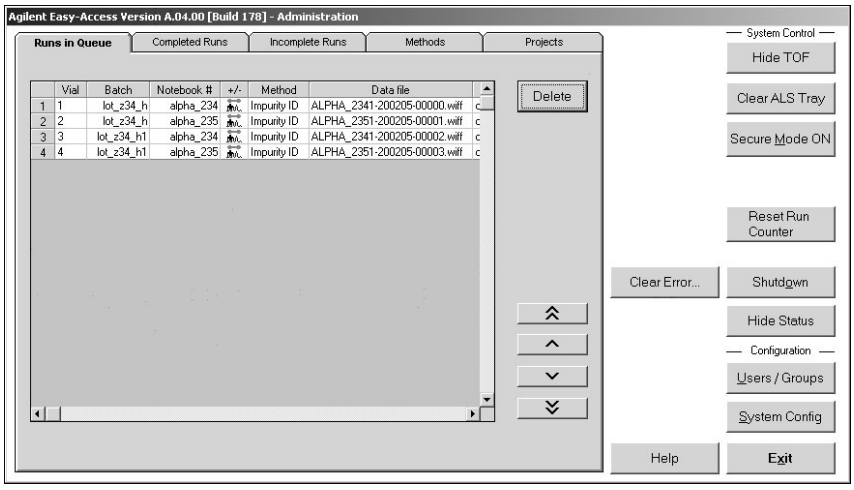

Figure 7
Main administration screen showing current sample queue.

Setup and administration

The system can be set up by a system administrator to match most laboratory protocols in areas such as security, terminology, methodology, and so on. Changing the setup is under password control. The first level of the administration software is shown in figure 7. This panel shows the current queue and allows the administrator, or other users if given the capability, to change the order the samples are run or delete them if desired. Here is where the administrator sets up methods, decides what other applications users can access, sets up new users and configures the overall system. The administrator establishes which of the instrument methods are available in Easy-Access and optionally, which groups may use the methods. The allowed range of injection volumes is also set up for each method. Figure 8 shows how an Easy-Access method is set up. Note that an acquisition method and a reporting (DA) method are combined to form a single Easy-Access method. The administrator can even choose to receive e-mail notification of system errors or low solvent state. An example of one of these messages is shown in figure 9.

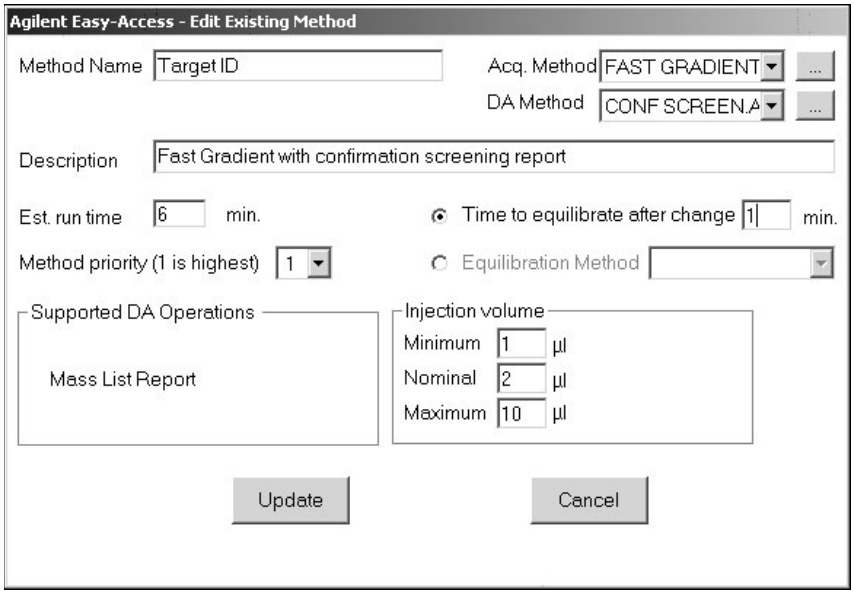

Figure 8
Screen showing editing an Easy Access Method.

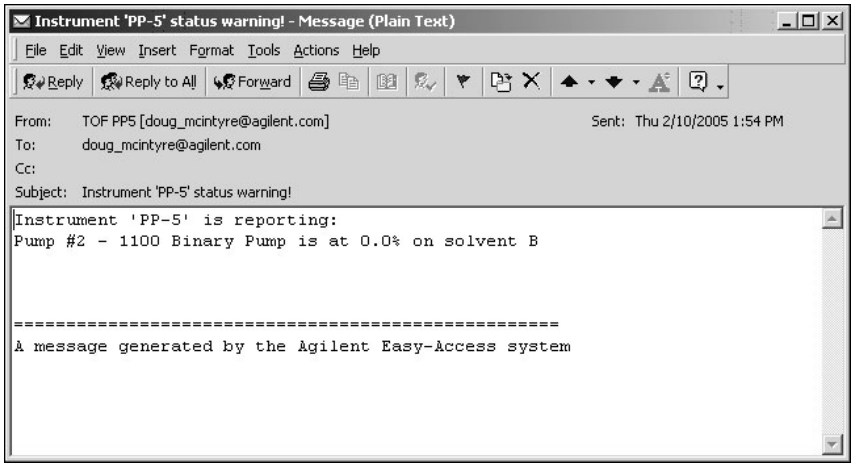

Figure 9
E-mail notification to system administrator of low solvent condition.

The system can be set up to work with submitters with a range of skill levels. For example, some users may be able to specify injection volume, move samples in the queue and have a wider range of methods while others may have much more limited capability. The maximum number of samples per user may also be established. Figure 10 shows the user setup screen.

The methods, instrument configuration, sample history and user account information are all maintained in a database. For multiple laboratories with several Easy-Access systems, the databases can be shared. This means user information, and so on, need only be set up once. In laboratories where tracking samples for accounting purposes is needed, the sample history can be exported to a file or database.

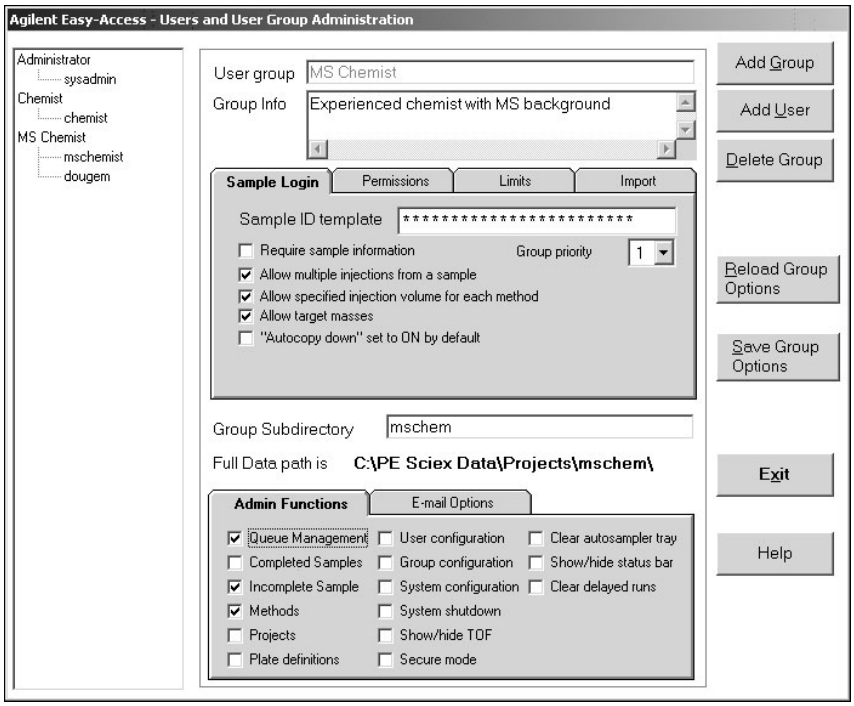

Figure 10
Easy-Access screen for setting up and determining user capabilities.

Conclusion

The Easy-Access software used with the Agilent LC/MSD TOF allows laboratories to obtain results requiring accurate mass measurement without requiring highly skilled instrument operators. This allows chemists to confirm the correct synthesis of their products and identify unknown compounds more easily. The common user interface for quadrupole and TOF systems makes it even easier to use in multi-instrument laboratories.

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