

Open Access High Resolution Accurate Mass Analysis using a Benchtop Orbitrap™ LC-MS system

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Lester Taylor¹, Helmut Muenster² and Karen Salomon¹

1 Thermo Fisher Scientific, San Jose, CA, USA

2 Thermo Fisher Scientific, GmbH, Bremen, Germany

Overview

Purpose: We have developed an instrument/software package which allows non-expert users to analyze and process ultra-high resolution accurate mass data in a walk-up, open access mode.

Methods: The software offers three distinct workflows for survey screening, target matching and target screening.

Results: The software can be used for easy set of data acquisition and reporting. Results are enhanced with Intelligent Elemental Composition calculations for added confidence.

Introduction

Open access LC-MS instruments have been used extensively for routine screening of target compounds and for compound identification/verification. Initially developed using low resolution single quadrupole LC-MS systems, several different types of open access instruments have been used including TOF and triple quadrupole mass spectrometers. We report the development of an ultra-high resolution benchtop Orbitrap LC-MS system. With its new open access software this LC-MS system is capable of producing unambiguous elemental compositions for users with little or no expertise in mass spectrometry.

Methods

Non-expert users can access pre-defined LC-MS methods and reports and are guided through a simplified sample log-in process. Analysis reports can be obtained in a variety of formats. Software allows the expert user to set up the LC-MS system and monitor usage, either while in the laboratory or remotely. The expert user has full access to the system to create user accounts, initiate sample processing, analytical method selection and results reporting.

Elemental composition calculations are used to generate chemical formulae for the compound of interest. A new algorithm is used to give a single high confidence elemental composition based on using information from accurate mass and isotope ratio abundances.

Background

An open access LC-MS laboratory enables scientists to get answers quickly, without waiting for a remote laboratory to send back results. Routine analyses are performed efficiently, leaving mass spectrometry experts more time to focus on solving the most challenging analytical problems. The Thermo Scientific Pathfinder software enables walkup access to the unique and powerful capabilities of the Exactive™ instrument, which routinely delivers ultra-high resolution and accurate mass data for every scan without the need for data averaging. Operating at a 10 Hz scanning frequency, the Thermo Scientific Exactive is fully compatible with U-HPLC technologies and ensures exact mass measurement for fast chromatography applications. Rapid polarity switching enables the detection of the widest range of compound types. Open access software offers ease of use and high selectivity and is ideal for core chemistry labs, universities by synthetic and medicinal chemistry groups.

Pathfinder™ Workflow

Pathfinder provides an intuitive interface for both the walk up and the experienced users (Figure 1a). The user mode allows the analyst to log in, select an analysis type from one of three choices (Figure 1b), load their samples, click "GO" and pick up their report. Pre-defined methods are used to produce automated reports for LC-MS analysis.

Pathfinder allows an administrator to set up the system and determine user access. The Administrator will set up the analytical methods available to the user. Administrators have access to data review and reprocessing as well as results reporting (Figure 1c). Administrators can also initiate an LC-MS analysis and track the status of the Exactive system in real-time.

Results can be configured for printed or electronic reports in pdf and other formats. Elemental composition results are included as well and reported with confidence values which identify a single candidate composition.

FIGURE 1. Pathfinder Software

- Welcome screen for user and administrator
- User selections
- Administrator options

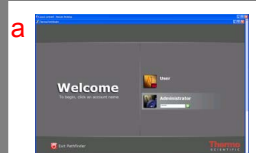
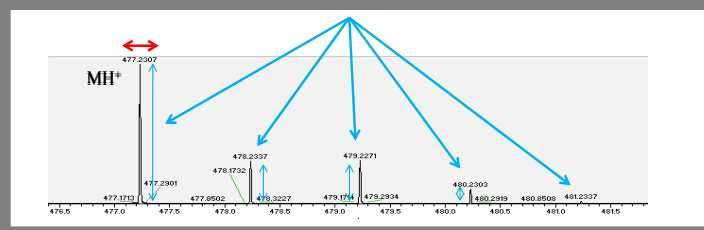


FIGURE 2. Intelligent Elemental Composition calculation (blue) compared to traditional formula calculation (red) around one exact molecular mass. The Intelligent Elemental Composition algorithm takes into consideration isotopic masses and their intensities.



Intelligent Elemental Composition Determination

The unambiguous determination of elemental compositions is essential for the identification of target compounds in numerous applications. The traditional approach to elemental composition calculation was based on one exact mass only. This resulted in a long list of elemental composition candidates. This list explodes with increasing mass, increasing mass tolerance and increasing number of elements that must be considered. High mass precision helps reducing the number of elemental composition candidates but at masses above 500 even a precision of 1 ppm results in too many candidates.

The Intelligent Elemental Composition determination uses isotope accurate mass data and ratios to calculate the chemical formula. A single mass – usually the mono-isotopic mass – of a measured isotope pattern is used to calculate all possible elemental compositions that lie within a tolerance window that is determined from the expected mass error of the measurement. Then the theoretical isotope pattern for each elemental composition candidate is calculated. In the next step the "Spectral Fit" between the theoretical and measured isotope pattern is calculated. The Spectral Fit is a number between 0% (where the patterns are completely different) and 100% (where the patterns are identical). The composition candidates are sorted in decreasing spectral fit order (Figure 3).

Intelligent Elemental Composition is enabled for the Survey Screening and Target Matching Workflows. The administrator will select the elements to include in the calculation and can adjust the mass tolerance used and the number of hits displayed.

FIGURE 3. Results of formula calculation for loperamide showing high fit factor and calculated and theoretical peak profiles compared to the measured profiles and a comparison to the number of results obtained from a traditional calculation (Qual Browser).

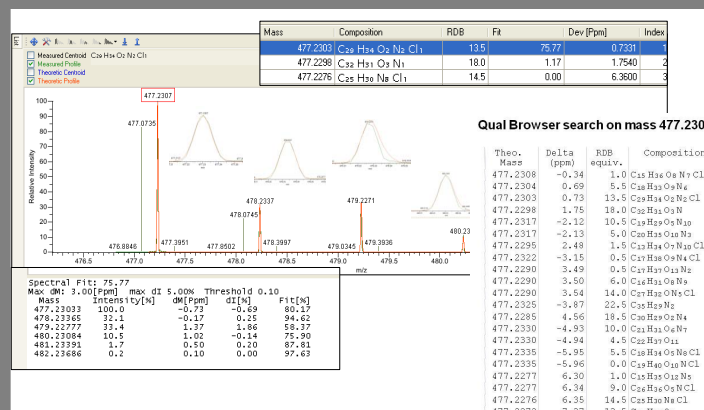
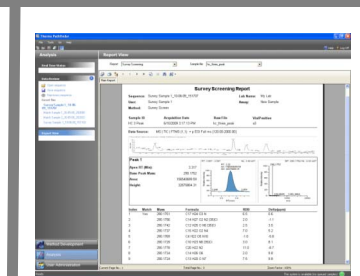


FIGURE 4a. Review of Survey Screening results showing the peaks found and the mass spectrum of the highlighted peak.



FIGURE 4b. Survey Screening report showing Intelligent Elemental Composition results for one of the peaks in the chromatogram.

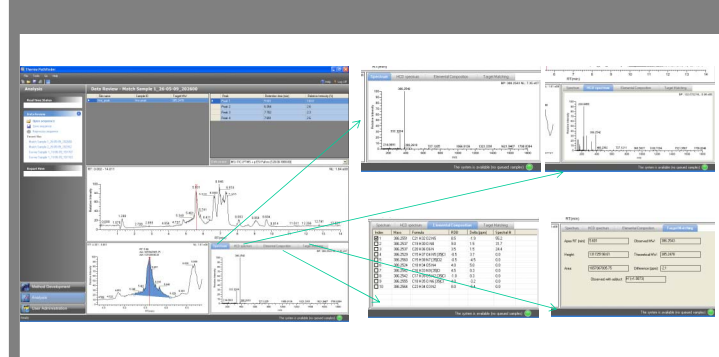


Results

Survey Screening is useful for those analyses having no prior knowledge of which compounds might be present in the sample. For survey screening analysis, Pathfinder scans the Exactive data file for chromatographic peaks and creates a report summary for each peak detected. Integrated peak areas are high lighted in blue making it easy to scan through the report to focus on peaks that have larger areas. Peaks can be manually re-integrated in the data review section. The report includes retention time, peak height and peak area for each detected peak, along with a mass spectrum from the peak apex and an HCD spectrum if available. Elemental composition results are included as well.

Target Matching performs a search for the presence of a specified molecular weight in a sample and is used to confirm the presence of the desired compound. The software checks for the MH+ adduct ion or other specified adducts for the target compound and it can also consider negative ions and adducts. The target matching workflow searches the chromatogram for a peak of specified m/z value within user-entered mass tolerance. For those peaks found within these criteria, Pathfinder performs an elemental composition calculation as a confirmation of the peak identity. Results reporting includes an HPLC chromatogram, full scan mass spectrum, HCD spectrum, elemental composition confirmation and details of the target matching (Figure 5).

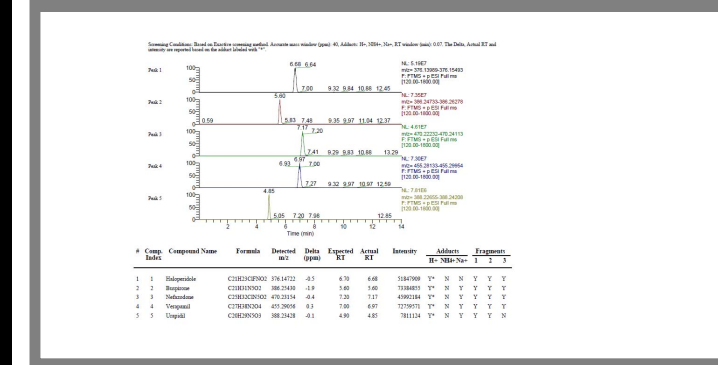
FIGURE 5. Results of a Target Matching experiment showing the full scan spectrum, HCD spectrum, elemental composition calculations and target matching parameters.



Target Screening analysis searches the detected masses in a sample against a user-defined library of accurate masses within a selectable narrow mass tolerance window. Searches can be narrowed by applying a retention time window, but this is often not necessary with the high resolution and accurate mass capability of the Exactive LC-MS and the option is left up to the user. An added feature of target screening is that up to three characteristic MSn fragments can also be included in the data base. Compound adducts are easily entered into the data base.

Samples are identified by comparing observed extracted full scan mass chromatograms and comparing the observed peak against the entries in the library. Results can be reported only for those compounds found or for all compounds in the data base. Additional adduct and fragment matching enhances confidence in the results. Reporting options include a summary report on all compounds (Figure 6) or a detailed report of each compound.

FIGURE 6. Results of a Target Screening experiment showing the use of HCD fragments as confirmation of matched compounds.



Conclusions

- Pathfinder software used with the Exactive LC-MS system provides confident, walk-up analysis of both simple and complex samples.
- Built-in workflows provide easy access to an ultra-high resolution LC-MS platform for compound identification and high throughput screening. With automated display of elemental composition, Pathfinder creates a high productivity environment for multiple users with a range of mass spectrometry experience.
- Intelligent Elemental Composition is used to process exact mass data as an added confirmation for target matching and for compound identification in survey screening. This algorithm reduces the set of candidate fits compared to traditional methods and also provides a confidence value to the reported composition.
- Future studies will employ new background subtraction algorithms to enhance the survey screening workflow for complex matrices.

Acknowledgements

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References

L.C.E. Taylor, R.L. Johnson, and R. Raso. "Open Access APCI Mass Spectrometry For Routine Sample Analysis." *J. Am. Soc. Mass Spectrom.*, 1995, 6, 387-393.

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