

Rapid Metabolite Identification using MetabolitePilot™ Software and TripleTOF™ 5600 System

Easily Find, Identify and Confirm Metabolites in Accurate Mass Data

Carmai Seto, Tanya Gamble and Hesham Ghobarah
AB SCIEX, Concord, Ontario, Canada

The ability to find, identify and confirm metabolites as quickly as possible is critical at multiple stages of drug discovery and development. Advancements in instrumentation have enabled the generation of very information rich raw data. New software to automate parts of the metabolite identification workflow holds the potential for considerable benefits to drug metabolism researchers. For example, accurate mass instruments such as Time of Flight (TOF) have the capability to provide both qualitative and quantitative information in a single analysis. However, the data analysis can be quite complicated and very time consuming if performed manually, especially if analog data was acquired along with MS and MS/MS data. Software can automate the process of finding, identifying and confirming metabolites in accurate mass data. In this technical note, we will describe how MetabolitePilot™ software and the AB SCIEX TripleTOF™ 5600 system can make the process of identifying and confirming metabolites more efficient than ever before.

MetabolitePilot™ Software: Automated Identification and Confirmation of Metabolites

MetabolitePilot software employs multiple powerful data processing algorithms to perform both non-targeted and targeted processing in parallel. In addition to generic peak finding, multiple mass defect filtering, isotope pattern matching, and finding metabolites based on common product ions or neutral losses can all be used simultaneously to efficiently find and identify metabolites. To harness the power of these algorithms, the software automatically calculates the most appropriate compound-specific processing parameters for a given compound. MetabolitePilot software automatically predicts potential cleavage metabolites based on the structure of the parent compound. It also automatically calculates mass defect filters for Phase I metabolites, including the parent drug, as well as Phase II and potential cleavage metabolites (Figure 1).

MetabolitePilot software makes the work of identifying and confirming metabolites more efficient with an easy to use integrated workspace that displays all relevant information. The software displays the analog, MS and MS/MS data associated

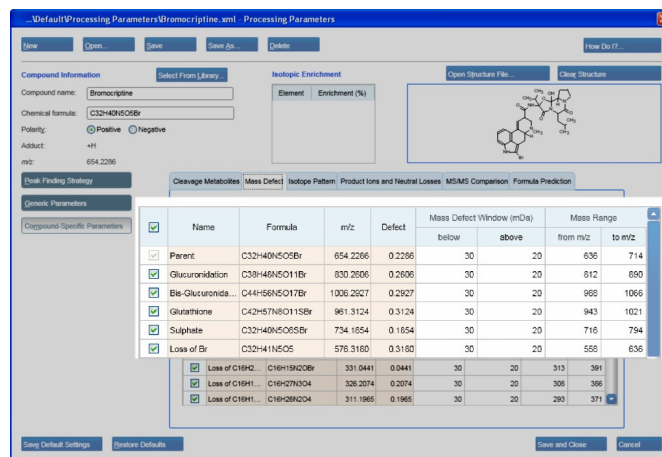


Figure 1. Multiple mass defect filtering setup in the processing parameters. MetabolitePilot™ software automatically calculates the mass defect filters for multiple metabolite classes using the parent structure.

with each metabolite in a single view (Figure 2). A confirmation score is also provided to help determine if a peak is a metabolite (Figure 3). To aid with confirmation of metabolites, the software enables faster comparison of metabolite and parent product ion spectra with a simple and effective display. The product ion spectrum of the parent drug is automatically compared with each metabolite and the results of the comparison are displayed in the MS/MS panel of the results workspace.

Key Features of MetabolitePilot Software for Metabolite Identification

- Intuitively supports the TripleTOF™ 5600 LC/MS/MS system's high resolution accurate mass metabolite identification workflows^{1,2}
- Metabolites are detected through a combination of powerful peak finding algorithms
- Automatic structure driven processing parameters
- Integrated MS/MS fragment interpretation and structure editor
- Multiple sample correlation function for time course studies and inter-species comparison of metabolites
- Simple, clear data reviewing enabled by the Results Workspace

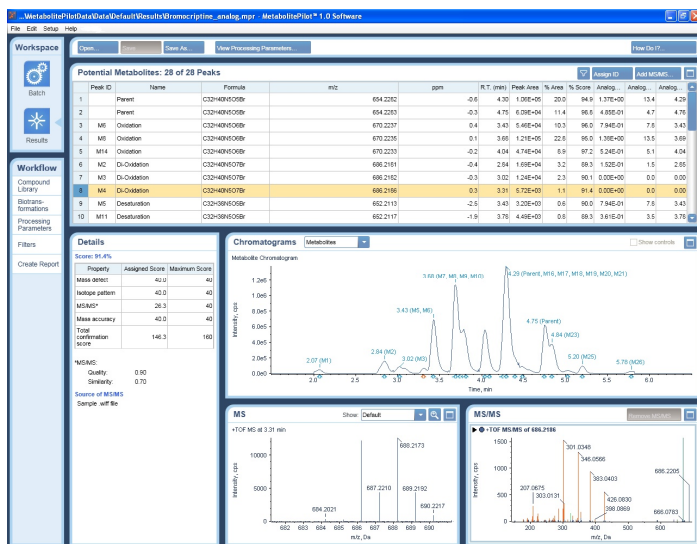


Figure 2. Results workspace of MetabolitePilot™ software All data is displayed in a single workspace for efficient data review.

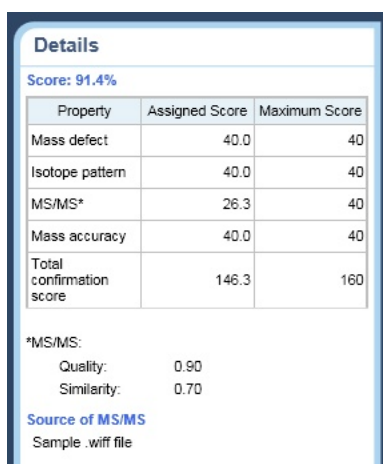


Figure 3. Detailed information about a confirmation score. Each potential metabolite is given a confirmation score to help determine if the peak is likely to be a metabolite.

Experimental Conditions

Sample Preparation: Bromocriptine was incubated in rat liver microsomes under oxidative conditions at 37° C for 1 hour, along with a control. The reactions were quenched by the addition of an equal volume of acetonitrile to the microsomal solutions then centrifuged at 14,000 rpm for 5 minutes.

Chromatography: The incubations were analyzed using a Shimadzu Prominence UFLC-XR LC system with UV detector

coupled to TripleTOF™ 5600 system. All analyses were performed using an Imtakt Cadenza CD-C18 (2 x 50 mm) 3μ, ODS column that was held at 40°C. Solvent A contained water with 0.1% formic acid and solvent B contained acetonitrile with 0.1% formic acid and the flow rate was set at 600 μL/min. The gradient is summarized in Table 1.

Table 1. Summary of LC gradient used to analyze the incubations

| Time (minutes) | Composition of Gradient (%B) |
|----------------|------------------------------|
| 0.0 | 10 |
| 0.5 | 10 |
| 0.75 | 20 |
| 5.5 | 40 |
| 6.5 | 60 |
| 6.8 | 90 |
| 6.9 | 90 |
| 7.0 | 10 |

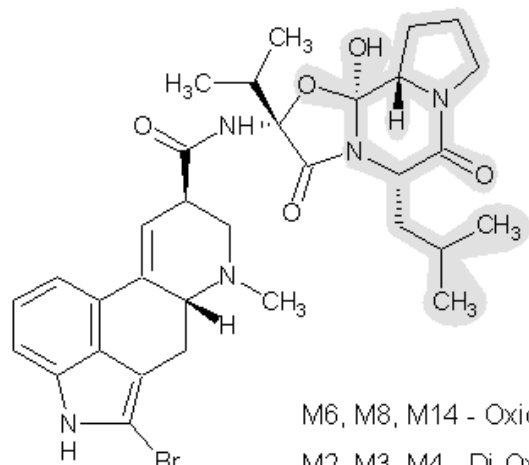
Analog Data: The Shimadzu UV dual wavelength detector was set to 302 nm.

Mass Spectrometry: The TripleTOF 5600 system method used for data acquisition consisted of a TOF MS survey scan followed by two IDA TOF MS/MS scans. Mass Defect triggered IDA with Dynamic Background Subtraction™ algorithm was used to collect the MS/MS data. External mass calibration was performed automatically using a calibrant delivery system.

Results

MetabolitePilot software was used to process the data acquired for the incubation. The software correlated the MS and UV data together in the results workspace, with the potential metabolites table indicating if a MS peak has a corresponding analog peak (Figure 2).

Figure 4 illustrates some of the structures proposed for some of the metabolites that were identified by the software. The identified peaks were confirmed as metabolites using the confirmation score provided by the software, the exact mass product ion spectra and the UV data.



M6, M8, M14 - Oxidation
M2, M3, M4 - Di-Oxidation
M5, M4, M23 - Desaturation

Figure 4. Proposed structures for some bromocriptine metabolites identified in a rat microsomal incubation.

Along with the interpreted spectrum of bromocriptine, structural elucidation of the metabolites was quickly achieved using the excellent mass accuracies achieved by the TripleTOF™ 5600 system. When comparing product ion spectra, common product ions between metabolites and parent drug (bromocriptine) indicate where metabolism has not occurred, while common neutral losses indicate where metabolism has occurred. The comparison process is made more efficient by the automated product ion comparisons and fully integrated automatic MS/MS fragment interpretation and assignment performed by MetabolitePilot™ software. Editing and storage of metabolite structures can be performed directly within the software. Interpretation and inter-sample correlation capabilities are covered in detail in reference (3).

An example of the product ion spectra used for structural elucidation in this study is shown in Figure 5; the spectrum for an oxidation metabolite (M6), a desaturation metabolite (M23) and bromocriptine are shown. Due to the presence of the common product ion m/z 426.0809, the sites of metabolites were determined not to be localized on the substructure represented by m/z 426.0809. There is no ambiguity in the elemental composition of the product ion due to the mass accuracies achieved by the TripleTOF 5600 system for this experiment.

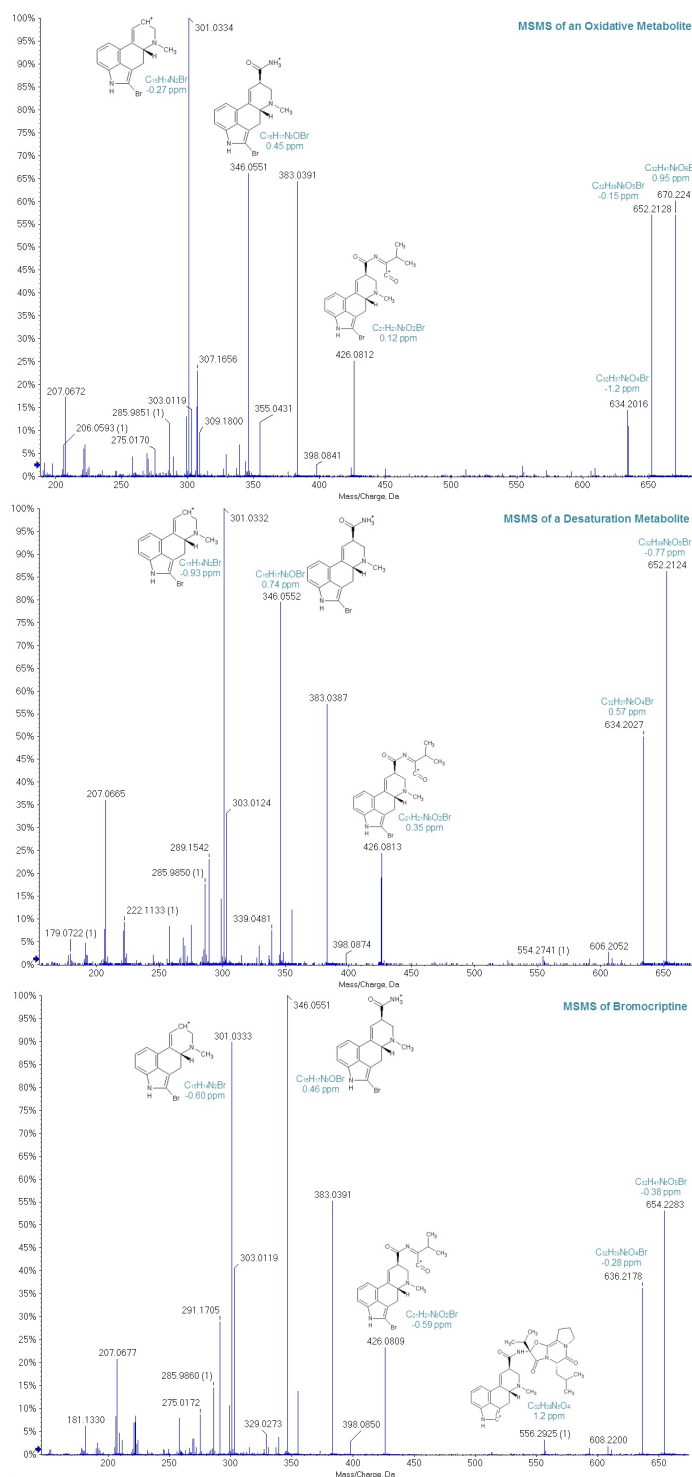


Figure 5. Product ion spectra of two metabolites and bromocriptine. Automated MS/MS comparisons and excellent mass accuracies allowed for rapid structural elucidation of metabolites.

Conclusions

The TripleTOF™ 5600 system with MetabolitePilot™ software effectively address the data analysis bottleneck in metabolite identification, from peak detection to structural elucidation.

- Efficiently find metabolites using powerful multiple peak finding strategies applied in parallel, such as non-targeted generic peak finding and mass defect filtering.
- Automatic generation of structure based processing parameters such as multiple mass defect ranges and potential cleavage metabolites.
- Efficient processing of multiple sample sets together in the Batch Workspace.
- Rapidly perform structural elucidation using integrated MS/MS fragment interpretation and assignment, all in a single user friendly workspace.
- Automatic multiple sample correlation of UV, MS, and MS/MS data, and plotting of response across samples for faster and easier evaluation of time course studies and inter-species comparison.

References

1. "Breakthrough Productivity for ADME Studies Using the AB SCIEX TripleTOF™ 5600 System", AB SCIEX Technical Note, Publication 0480110-01
2. "Simultaneous Pharmacokinetic Profiling and Automated Metabolite Identification using the AB SCIEX TripleTOF™ 5600 System and MetabolitePilot™ Software", AB SCIEX Technical Note, Publication 1270210-01
3. "Solving Bottlenecks in Metabolite ID Data Analysis With MetabolitePilot™ Software", AB SCIEX Technical Note, Publication 3610211-01
4. To download a trial version of MetabolitePilot™ software, please visit:
<http://www.absciex.com/Products/Software/MetabolitePilot-Software>

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Headquarters

353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

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