

An Intelligent UPLC/TOF MS Workflow for Metabolite Identification Using a Software Tool including C-Heteroatom Cleavages and Automatic Generation of Mass Defect Filters

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INTRODUCTION

The major bottleneck in metabolite identification is data processing because it is still mainly a manual process. The biggest challenge for automated data processing is the large number of false positives which may be generated. A powerful tool to help for the removal of these false positives is to use a mass defect filter (MDF) ^{1,2}. Optimizing the MDF on a compound specific basis is an important step, since different compounds will give rise to different metabolic cleavages and may not exhibit a predictable fixed linear range of mass defect, such as drugs containing S, Cl, or Br. We have previously presented a proof of concept approach using a simple algorithm which can quickly generate an intelligent compound-specific mass defect filter³.

In this work, we present the integration of this algorithm into a fully-featured metabolite identification package workflow (Figure 1). This algorithm (Dealkylation tool) allowed us to query a particular drug upfront to generate a list of C-heteroatom and heteroatom-heteroatom cleavages. This information was used to generate an automatic filter with adjustable limits depending upon whether Phase I or II metabolites are selected.

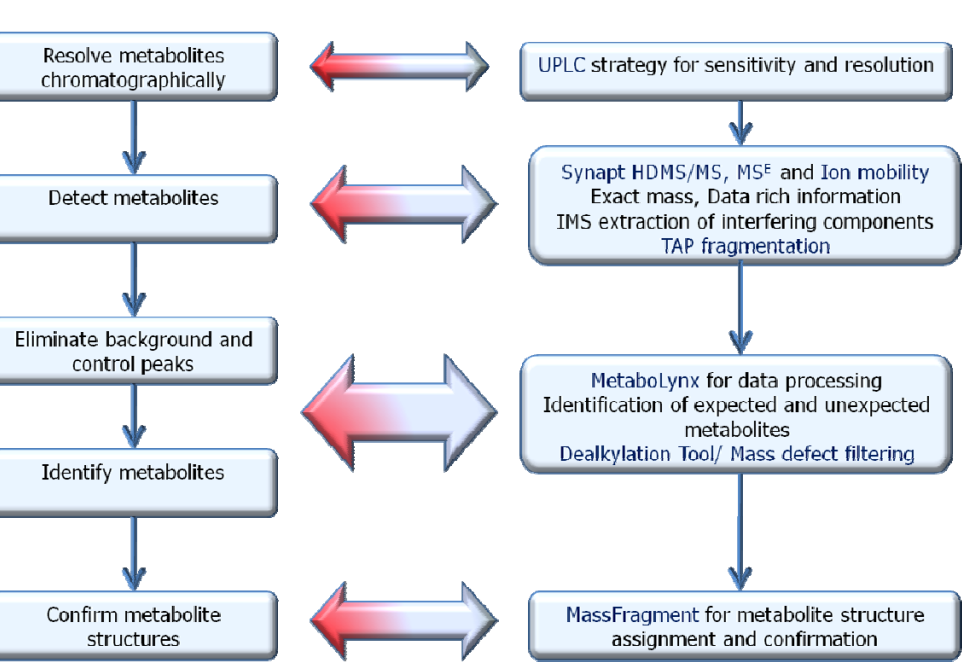


Figure 1. UPLC/MS^E Metabolite Identification Workflow.

METHOD

Samples

Nefazodone (and Indinavir) was incubated with rat liver microsomes at 10 μM at 37 °C, in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing NADPH regenerating system and GSH at 5 mM. The reaction was terminated after 90 minute with 2 volumes of cold acetonitrile to 1 volume of sample. Then, the sample was centrifuged at 13,000 rpm for 15 minutes and the supernatant was diluted 1/2 with Water +0.1 % formic acid. Finally, the supernatant was injected directly to the UPLC-Synapt HDMS system for analysis.

LC-MS Methodology

LC-conditions:
LC System : Waters Acquity UPLC™
Column: Acquity BEH C18 Column 100x2.1mm id, 1.7μm
Column Temperature: 45 °C
Mobile phase A: 0.1 % Formic acid; B: Acetonitrile.
Flow rate: 0.6 mL/min
Gradient: 98%A – 40% A in 8 mins, ramp to 40-0%A in 1.5 min before returning to 98% A for re-equilibration
Injection volume: 5 μL

MS-conditions:
Mass Spectrometer: Waters Synapt HDMS™
MS acquisition range: 50-900 Da
Mode of Operation: + ion mode ESI
Lock Mass: Leucine Enkephalin at 200 pg/mL

Processing software

MetaboLynx was used for the MS^E data mining^{4,5} and peak detection of putative metabolites (Figure 2)

MassFragment was used for structure elucidation

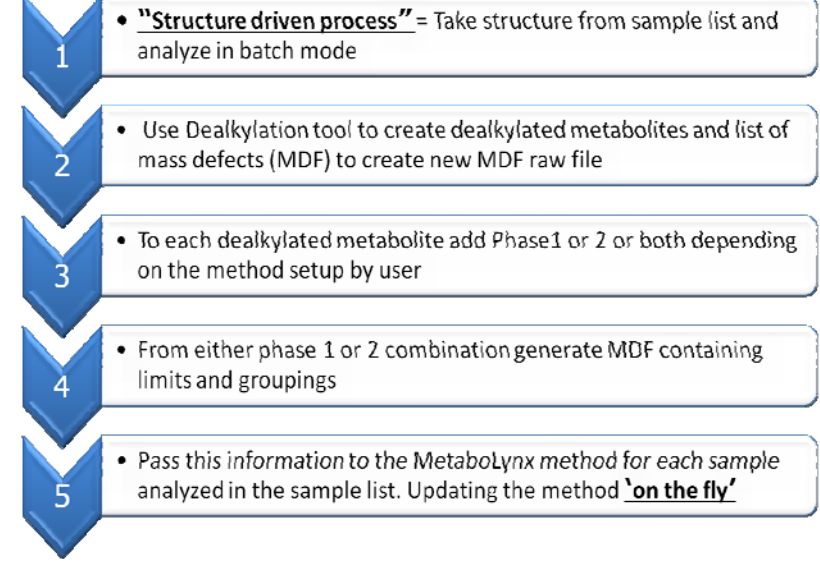


Figure 2. MetaboLynx Chemically Intelligent Workflow.

MASS DEFECT FILTER (MDF)

It can be utilized to remove false positives and improve the metabolite id workflow combined with prior knowledge of the mass and its decimal places (elemental composition). Mass defect can either be positive (larger than the nominal mass) or negative (smaller than the nominal mass). For example, The mass defect for H is +0.0078 with 1.0078 as its exact mass, and the mass defect for OH is –0.0051 with 15.9949 as its exact mass.

Mass defect filter (MDF) is a post-acquisition data filtering technique that is set based on the mass defect of the parent drug and its metabolites. The

rationale and assumption supporting the MDF is that the majority of metabolites only has small shift in their mass defect when comparing with the parent exact mass.

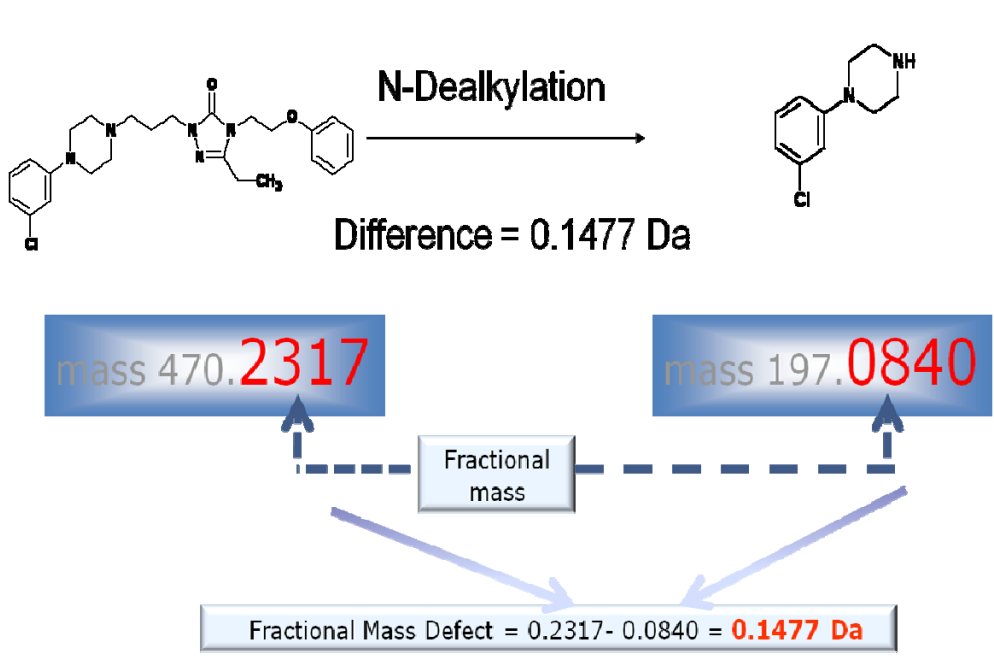


Figure 3. Mass defect shift after N-dealkylation occurred for nefazodone.

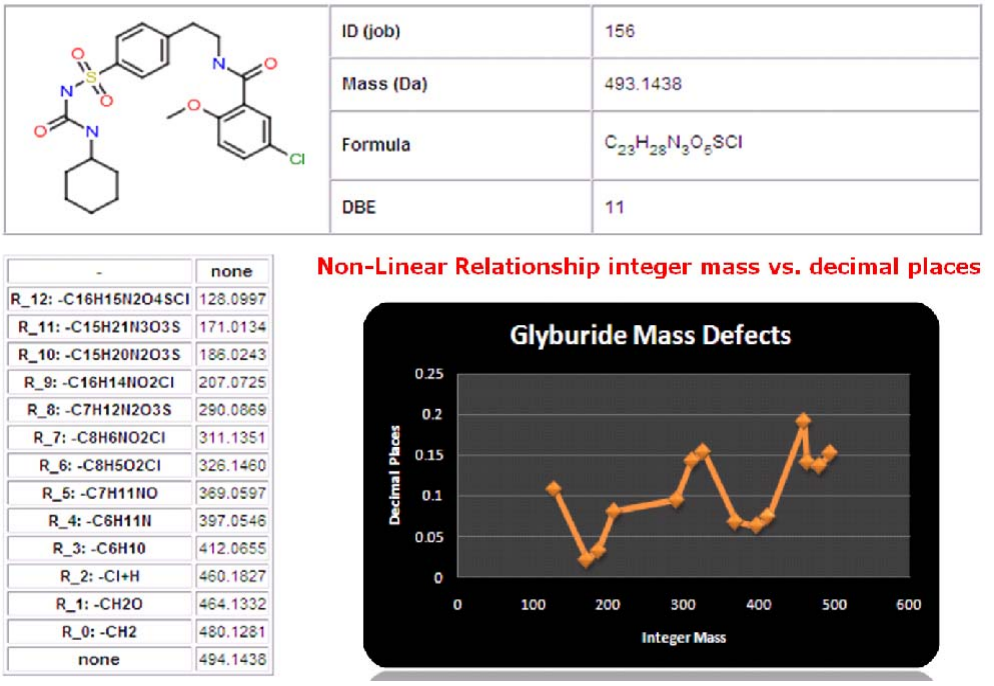


Figure 4. Mass defect shift after dealkylation occurred for Glyburide.

Applying the MDF technique allows users to capture and identify metabolites from complex matrices in a rapid and effective manner.

However, applying the MDF can be challenging and very much compound specific. First, the mass defect may have rather large significant shifts when a dealkylation occurred for parent drug prior to the formation of metabolites. (example shown in Figure 3). secondly, the mass defect shifts are not linear to their integer masses (example shown in Figure 4 for Glyburide).

MDF in MetaboLynx

In the UPLC/MS^E metabolite identification workflow shown in Figure 1, the MDF is applied at the initial stage during the MetaboLynx data processing. Once the user set up the MetaboLynx method, the parent drug structure is first automatically transferred to the dealkylation tool to identify metabolic cleavages. Upon identifying the major

dealkylated fragments, their corresponding MDFs are automatically grouped with expected biotransformations. The MDF's are calculated based on their individual metabolic cleavages and biotransformation addition to each metabolic cleavage and parent. Having done this, MetaboLynx can generate mass defect filtered chromatograms for control and dosed samples (.MDF files). The next step is for MetaboLynx to go through its regular metabolite identification procedure using the mass defect filtered chromatograms, with the aim of having a much more reduced list of unexpected metabolites.

RESULTS

Figure 5 shows how the mass defect filters are automatically set in MetaboLynx. Because of the big fluctuation of the mass defect as a result of the dealkylation, multiple automatic mass defect filters were set for the metabolite identification. Figure 5, 6 and 8 also shows the automatic generation of metabolic cleavages for Nefazodone and Indinavir. A comparison of the number of Nefazodone and Indinavir metabolites identified using Linear fixed MDF and Intelligent MDF is shown in Figure 7 and Figure 9 respectively.

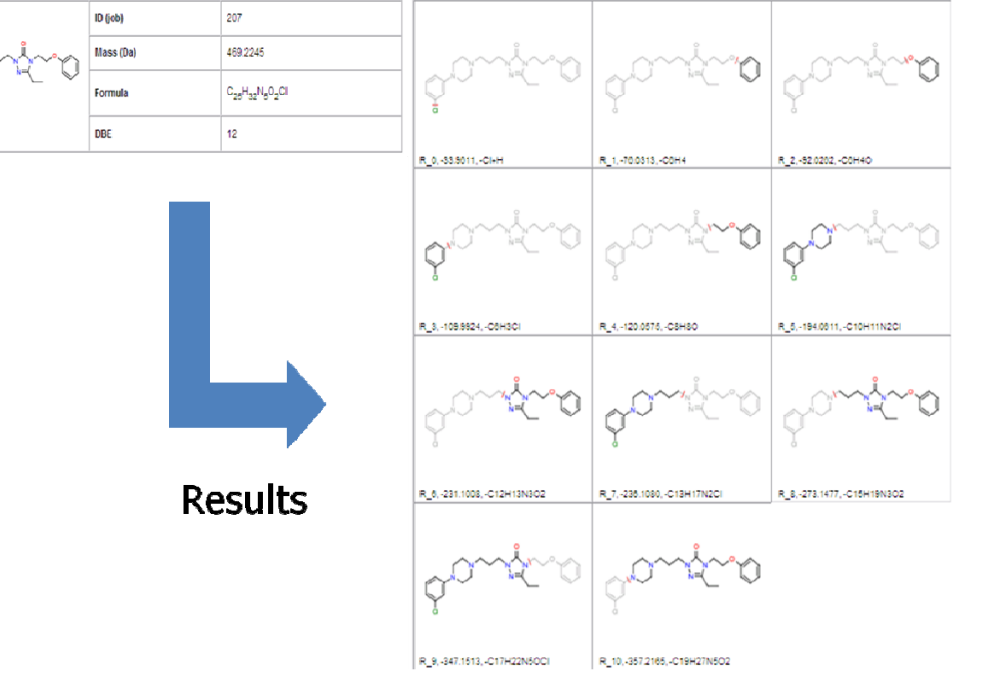


Figure 5. Automatic generation of metabolic cleavages for Nefazodone.

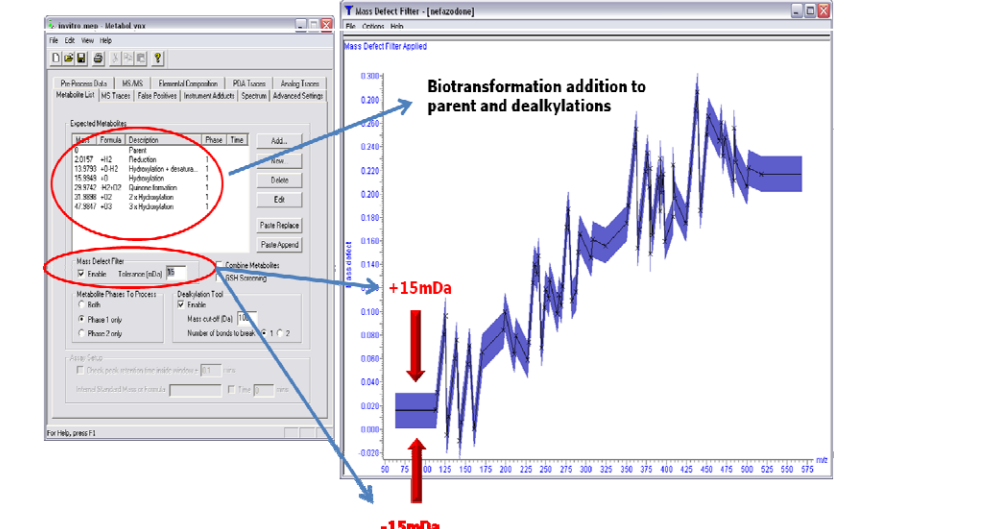


Figure 6. Automatic generation of metabolic cleavages and Mass Defect Filters for Nefazodone.

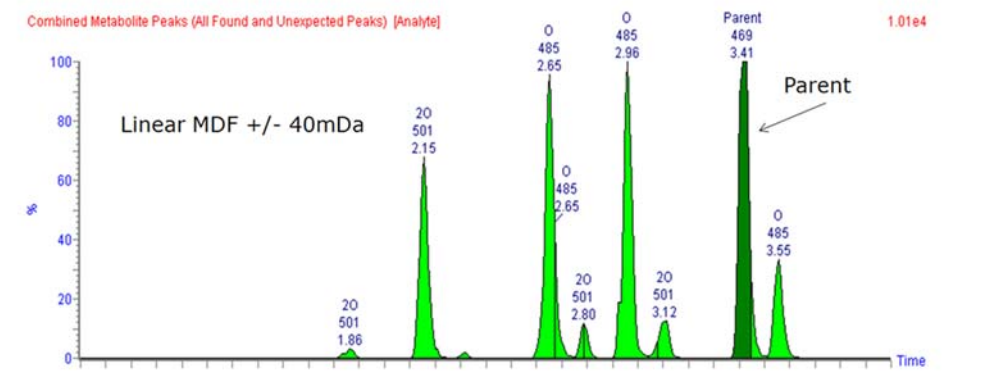


Figure 7. Nefazodone metabolites comparison using Linear fixed MDF (top) and Intelligent MDF (bottom).

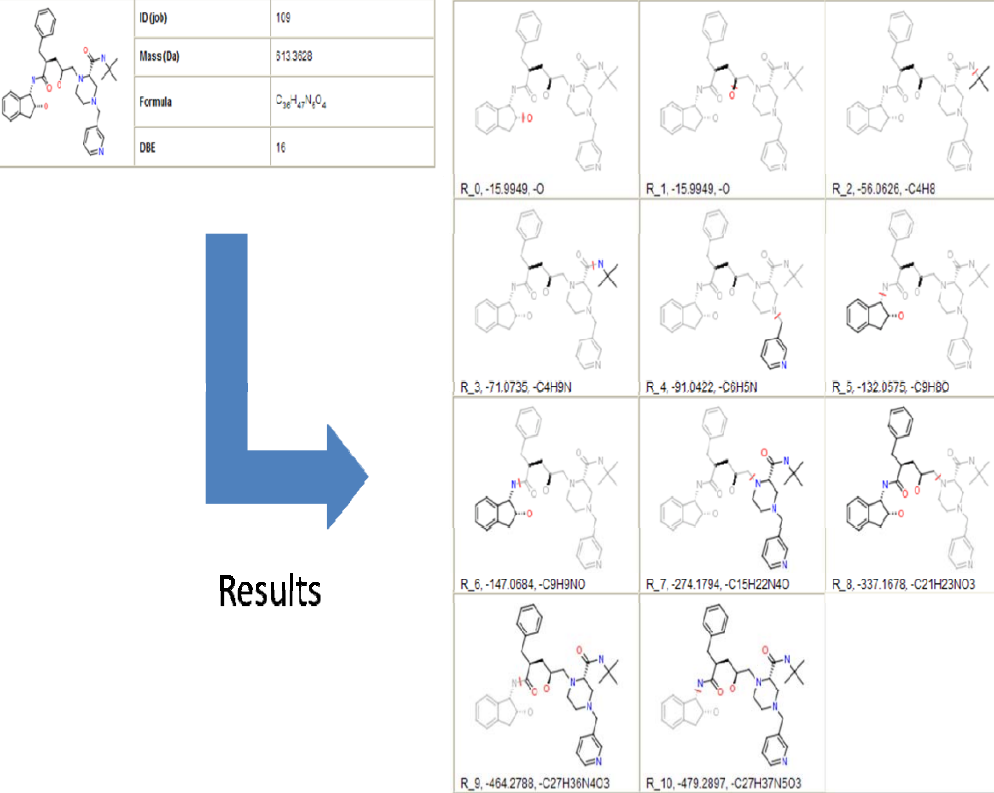


Figure 8. Automatic generation of metabolic cleavages for Indinavir.

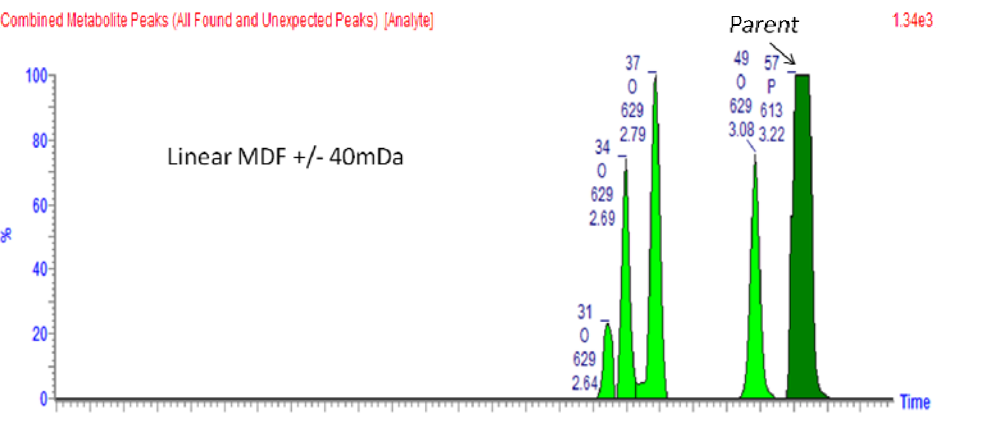


Figure 9. Indinavir metabolites comparison using Linear fixed MDF (top) and Intelligent MDF (bottom).

CONCLUSION

With the use of the dealkylation tool in combination with the mass defect filter we were able to rationalize the expected and unexpected metabolites more efficiently and minimize the number of false positives. From this initial step it was possible to generated an extensive expected metabolite list 'on-the fly'. The unexpected metabolites such as ring contractions or formations are also possible to detect since we allow for a confidence limit window for the MDF which will cover such biotransformations.

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