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Automated Software Tools to Localize the Site of Biotransformation

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

To automate the determination of the site of metabolism of a drug compound using exact mass product ion information and innovative software tools.

Benefits

MS^E data contains both precursor and product ion information for every detectable species in the sample and allows automatic comparison of the product ion spectra between metabolite and parent.

Introduction

Nefazodone's primary use is to treat depression in adults. Here we investigate the oxidative metabolism of nefazodone. In particular, para-hydroxynefazodone has been shown to produce reactive quinoid intermediates in human liver microsomes1, which may be responsible for reported hepatotoxicity issues that led to withdrawal of the drug from the market in 2004.

Metabolite Identification is not a trivial task. It requires a scientist to identify the major metabolites formed, perform targeted analysis to generate additional data, and finally elucidate the structures from the raw spectral data. This process can require many hours of manual interpretation, even when performed by experienced scientists. Tools that facilitate these complex interpretation tasks ultimately allow this knowledge to impact the decision making processes earlier in the process.

An example of this occurs in the early stages of drug discovery when efficient localization of the site of metabolism can provide medicinal chemists with the critical information needed to design better drug candidates in a meaningful and short timeframe.

In this technology brief, we demonstrate a single injection UPLC/MS^E approach to generate a comprehensive dataset and the use of software tools to automatically localize biotransformations.

Results and Discussion

Human liver microsomes spiked with 10 μM nefazodone were incubated for 0 and 60 min at 37 °C. Samples were quenched with one volume of acetonitrile + 0.1% formic acid and centrifuged. Supernatants were analyzed in positive ion mode using a UPLC/MS^E acquisition method.

5 μL of sample was injected onto an ACQUITY UPLC BEH Column (1.7 μm, 2.1 x 50 mm), and run with a 2 min gradient at a flow rate of 0.7 mL/min. The mobile phase consisted of 0.1% ammonium hydroxide (A) and methanol (B).

MS^E data were processed and analyzed using MetaboLynx XS v2.0 Software. The first step in identification of metabolites was the interrogation of full scan exact mass information. The built in chemical intelligence in MetaboLynx XS v2.0 Software automatically predicts parent-specific metabolism, while maintaining vigilance in the search for unexpected metabolites. The results for human microsomal Met ID are shown in Table 1. Table 1 shows that two hydroxylation (+15.9949 Da) metabolites have been detected. Confirmation of this assignment and elucidation of their structure require additional information. Features were aligned to remove unrelated background and co-eluting fragment ions, which provided clean and accurate fragment spectra. It was possible to confirm the biotransformation, and infer at which point modification in the structure occurred.

Metabolite name	Formula	*m/z* found	PPM	Time
Hydroxylation-$C_{15}H_{19}N_3O_2$ (R_13)	$C_{10}H_{13}ClN_2O$	213.079	-0.2	0.56
3 x Hydroxylation + Desaturation-$C_{10}H_{11}N_2Cl$ (R_10)	$C_{15}H_{19}N_3O_5$	322.140	-0.5	0.56
2 x Hydroxylation + Desaturation-$C_{10}H_{11}N_2Cl$ (R_10)	$C_{15}H_{19}N_3O_4$	306.146	0.5	0.61
Hydroxylation-C_6H_3Cl (R_5)	$C_{19}H_{29}N_5O_3$	376.235	0.4	0.76
Parent-$C_{15}H_{19}N_3O_2$ (R_13)	$C_{10}H_{13}ClN_2$	197.085	0.9	0.92
Parent-$C_{13}H_{17}N_2Cl$ (R_12)	$C_{12}H_{15}N_3O_2$	234.125	5.0	0.82
2 x Hydroxylation + Deethylation	$C_{23}H_{28}ClN_5O_4$	474.191	0.7	0.75
Hydroxylation + debenzylation	$C_{19}H_{26}ClN_5O_3$	408.181	1.4	0.80
Parent-C_6H_3Cl (R_5)	$C_{19}H_{29}N_5O_2$	360.240	-0.6	0.90
Hydroxylation + debenzylation-Cl+H (R_1)	$C_{19}H_{27}N_5O_3$	374.219	-0.8	0.92
Hydroxylation + Deethylation	$C_{23}H_{28}ClN_5O_3$	458.196	0.3	0.95
2 x Hydroxylation	$C_{25}H_{32}ClN_5O_4$	502.223	1.2	1.03
2 x Hydroxylation	$C_{25}H_{32}ClN_5O_4$	502.223	2.0	1.08
Hydroxylation	$C_{25}H_{32}ClN_5O_3$	486.228	1.1	1.20
2 x Hydroxylation	$C_{25}H_{32}ClN_5O_4$	502.225	5.0	1.26
Hydroxylation	$C_{25}H_{32}ClN_5O_3$	486.226	-2.6	1.46
Parent	$C_{25}H_{32}ClN_5O_2$	470.231	-2.9	1.59
Hydroxylation + Desaturation	$C_{25}H_{30}ClN_5O_3$	484.210	-2.7	1.61

Table 1. Metabolites automatically identified above a 1% threshold (by MS area).

MS^E data from the parent compound and the metabolite were automatically compared to determine the site of metabolism in a fully interactive spectral viewer, as shown in Figure 1. This information is displayed as a heat map, as shown in Figure 2, with scoring ranging from 0 to 100. A score of 100% (red) confirms that every identified fragment supports the fact that the biotransformation occurred at that specific site, while a score of 0% (black) indicates that none of the data support an assignment in this region of the molecule.

Figure 2 shows this scoring for the two detected hydroxylations of nefazodone. The heat map shows the biotransformation occurring on different parts of the molecule. The earlier eluting metabolite is designated as an aromatic hydroxylation, the later as an aliphatic hydroxylation.
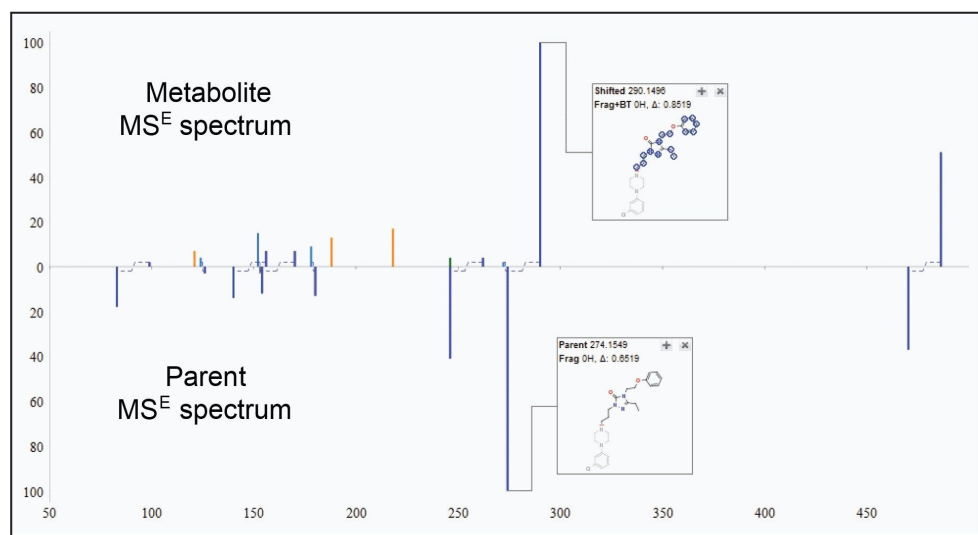
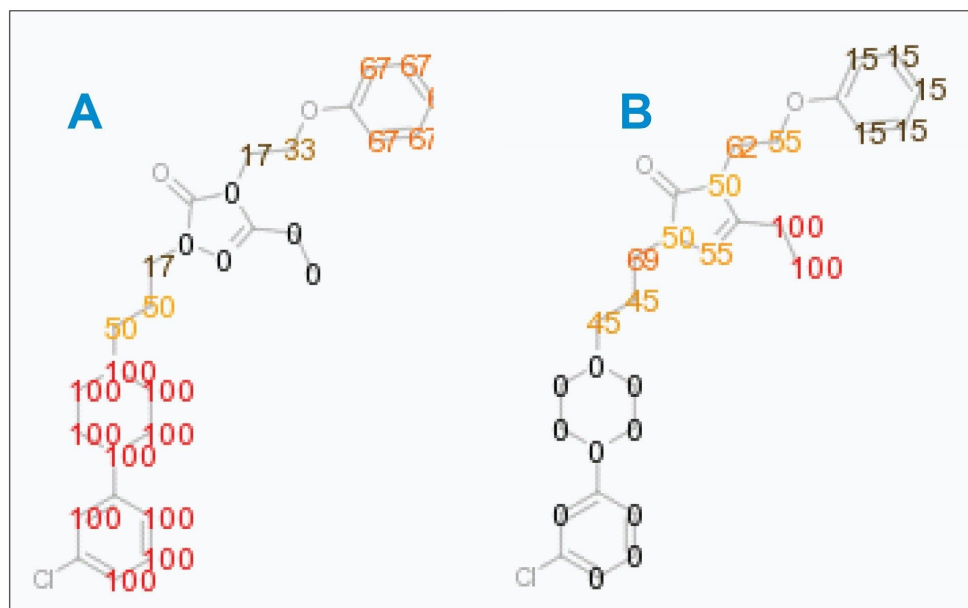Figure 1. Fully interactive spectral analysis for localization of the site of metabolism.

Figure 2. Automatically generated heat maps that show the evidence for the site of metabolism for the two hydroxylation's of nefazodone at A) 1.20 min B) 1.46 min.

Conclusion

We have demonstrated the use of a generic MS^E data acquisition and automated methodology for the determination of metabolic soft spots. These tools, in addition to the existing metabolite identification workflow, continue to redefine the quality and quantity of information, which is easily accessible from a single acquisition. This method can facilitate a more efficient transfer of knowledge among disparate groups, and can ultimately drive the drug discovery process forward.

References

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