# Automatic calculation and layout of MS/MS fragmentation pathways

ISMAEL ZAMORA<sup>1</sup>; Blanca Serra<sup>2</sup>; Luca Morettoni <sup>2</sup>;

<sup>1</sup>Lead Molecular Design, S.L., Sant Cugat del Valles, Barcelona; <sup>2</sup>Molecular Discovery, Ltd London, UK

## Introduction

The fragmentation pathway of a compound is part of the in-depth analysis of the compound behavior in the Metabolism Identification studies. This pathways help to understand not only the structural assignment of the compound itself but are also used to assign the observed peaks in the drug related materials detected in the experiment analysis, and therefore are important for the structure elucidation of those peaks.

In the present work an automatic way to produce a fragmentation pathway of a product of interest is presented.

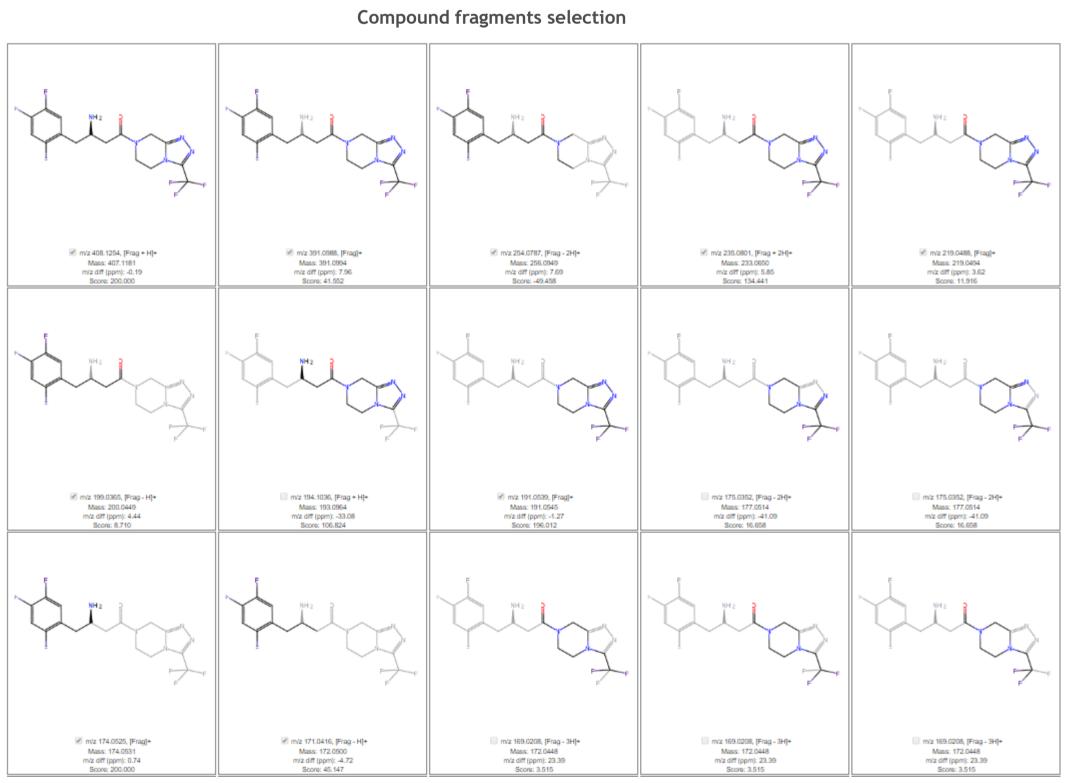
The system uses the MassMetasite and WebMetabase suite to analyze the HPLC-HRMS data of the product and assign compatible structures to the fragments observed in the compound analysis.

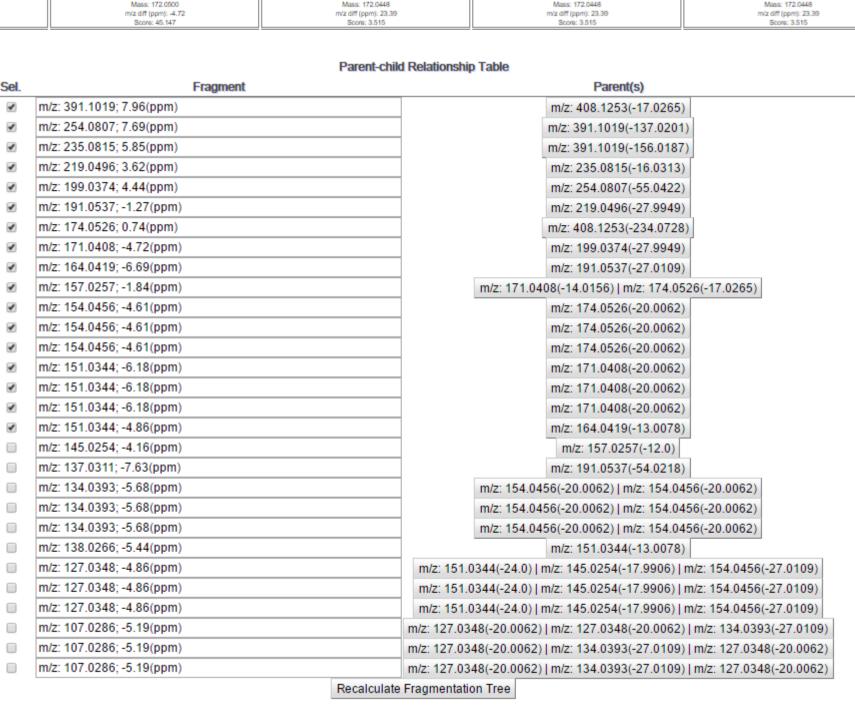
Then the fragments are layouted automatically in the fragmentation tree using their structural assignment.

## Results

The automatic layout of fragmentation pathways using the structural assignment of the compound fragments proposed by MassMetasite is a useful tool for the Metabolism identification scientists. It helps to quickly establish the structural relationship between the fragments of the compound under study, and thus to see if those relationships are maintained or lost in the related metabolic derivatives.

The tool proposes in just a click a pathway that can be refined and modified under the expert criteria, and then saved in the database for further reference.





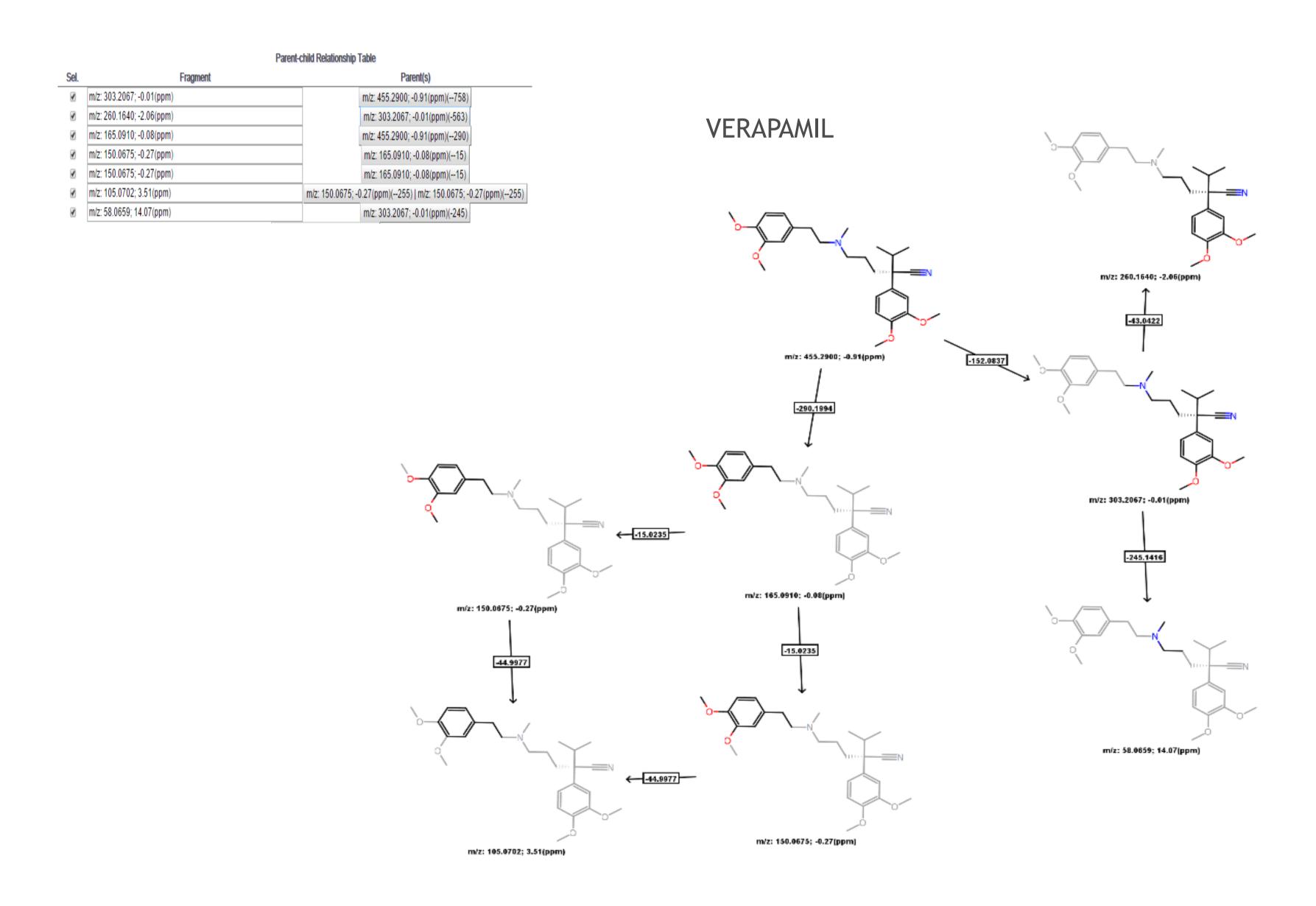
# SITAGLIPTIN SITAG

### Methods

Taking HPLC-HRMS raw data of a Metabolic experiment, and using MassMetasite to process it a structural assignment of the fragments of the parent compound MSn spectra is obtained. Using this data, and the mapping of the atoms between the compound and the different fragments the tool included in WebMetabase proposes automatically a fragmentation pathway.

This proposed pathway is not based on reaction monitoring data, and thus it is just a structure based approach, but it is a help to the metabolism identification studies to stablish if those more deep experiments or ways of analysis are needed.

The proposed pathway can be modified by the scientist both in the spatial layout, the fragments to take into account and the parenting of each of the fragments, and the result can be saved as a final image in the database for further reference. The image includes the molecular ion structure, the structures of the fragments with their m/z and the mass-change between fragments when a relationship (arrow) is drawn between fragments. In a first stage the proposed pathway can be rearranged or the parenting relationships can be revised before re-plotting the pathway image, then those images can be saved in the database for further reference.



## Conclusions

- It is possible to create automatically fragmentation pathways of compounds analyzed using the MassMetasite system where the MSn data of the compound of interest is analyzed and structures assigned to the different peaks.
- The system is limited to the fragmentation rules applied by the program.