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Metabolic soft spot identification in early drug discovery by Mass-MetaSite in comparison to manual interpretation of MS/MS spectra and NMR spectroscopy

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ABSTRACT

In drug discovery soft spot identification studies are performed to support the design of metabolically stable compounds. A limited factor for enhanced throughput is the time intensive manual interpretation of mass spectral data. The software Mass-MetaSite was evaluated as valuable tool for fast, automatic soft spot identification for a majority of compounds. Time of experts could be saved for those cases where Mass-MetaSite could not provide a structure and for complex structural assignments.

CONCEPT

For the design of metabolically stable molecules, information on metabolic soft spots of potential drug candidates is required as early as possible in the drug discovery process to support medicinal chemistry. Typically *in vitro* studies are performed and analyzed with LC-MS/MS. Commercial software products support the process, but only Mass-MetaSite (Molecular Discovery) combines the aspects of identification of drug related material, *in silico* prediction of MetaSite and rationalization of mass spectral fragment ions and batch processing.

In this thesis the performance of Mass-MetaSite was evaluated by comparison of the automatic structural assignments of metabolite structures with assignments retrieved from manual inspection of MS/MS spectra and for selected metabolites with exact structures from NMR structure elucidation. To demonstrate the value of using Mass-MetaSite on a regular basis for soft spot identification, compounds from discovery projects were analyzed with Mass-MetaSite in parallel to the standard soft spot identification process based on MS/MS used at Roche.

RESULTS

A dataset of 26 compounds (9 marketed and 17 Roche internal discovery compounds) were analyzed.

Identification of drug related material

On the level of peak detection, Mass-MetaSite identified 85% of metabolite peaks that were also extracted manually. In 15% the software failed mainly due to low peak intensity.

Comparison of assignments of Mass-MetaSite and MS/MS

Mass-MetaSite mimics structural elucidation from MS^E spectral information.

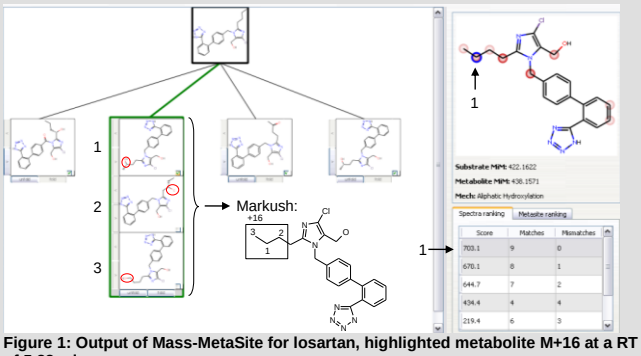

Figure 1: Output of Mass-MetaSite for losartan, highlighted metabolite M+16 at a RT of 5.33 min

The manual interpretation of MS/MS data often results in Markush-like structures. The software provides ranked definitive structures, which can be merged together to result also in Markush structures. Defining general rules which proposals to include to the Markush structures could not be achieved. The first rank structural proposal has a thorough benefit with regard to soft spot identification, if the suggested position is within the Markush structure.

The 1st first rank structural proposals of Mass-MetaSite were compared to Markush structures from manual interpretation from MS/MS spectra. For 79% of the metabolites (42 of 53), Mass-MetaSite provided structure proposals, 21% were not detected (8) or not identified (3). From all Mass-MetaSite proposals, 74% were in agreement with the manual approach (31 of 42), which means within the manual Markush structure, exact (cleavage metabolites) or better.

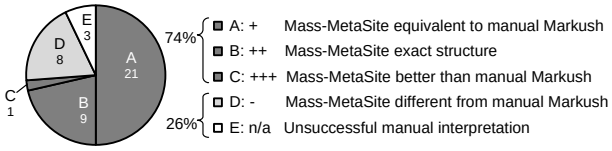

Figure 2: Structural assignment of Mass-MetaSite first rank proposals compared with Markush structure from manual expert interpretation. Mass-MetaSite assigned structures to total 42 metabolites.

Comparison of assignments of Mass-MetaSite and NMR

First rank structural proposals from Mass-MetaSite were only for two of ten metabolites assigned correctly, resulting from major dealkylations. However, for seven of ten metabolites the first rank was within manual Markush structure, which would not have provided further information in addition to the Mass-MetaSite result.

The main reasons for differences between Mass-MetaSite and the manual interpretation were different fragments in MSE and MS/MS raw data, which was confirmed by reprocessing of MS/MS data with Mass-MetaSite, where structure assignments were improved in five of ten cases.

Comparison of Mass-MetaSite approach with current approach

Six research compounds of current active Roche projects were analyzed, and 70% of metabolites were found by both approaches. The timeframe for the strategy with Mass-MetaSite was significantly less than for current expert interpretation.

CONCLUSIONS

To inform chemists on soft spots of molecules basic information on the major metabolites formed *in vitro* is sufficient in early stages of drug discovery, rather than having definitive metabolite structures. In contrast to the manual approach, the software approach requires little expert interaction and large data series can be run mostly unattended. Using Mass-MetaSite would allow to significantly reduce assay cycle time and to increase the throughput of tested compounds.

Mass-MetaSite is based on MS^E data and does not depend on a second injection for the acquisition of MS/MS spectra. But MS^E was less specific than MS/MS and the main reason for misinterpretations of the software.

Mass-MetaSite has the potential to enhance the process of soft spot identification and to contribute to an efficient interaction of biotransformation expert groups and chemistry teams to improve the design of new drugs.

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