MetaSense™: A Software Solution for Accelerating *in vitro* Investigations

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Abstract

The identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process is encouraged by regulatory authorities [1]. This comprises (i) a comparison of the metabolic patterns and examination of potential species differences, (ii) the identification of enzymes contributing to the metabolism in humans (P450, UDP-glucuronosyltransferases, etc.), (iii) testing metabolic activation [2, 3] as well as (iiii) determination of the *in vitro* metabolic stability to predict the human clearance, termed the *in vitro* $t_{1/2}$ approach [4, 5], which is nowadays routinely examined in a high through put manner at the pharmaceutical companies [6]. While sample preparation and data acquisition can be automated, data analysis (also “data mining”) and documentation becomes easily a bottleneck. Here we report a case study outlining the findings based on an examination of a parent compound studied across three different species and multiple incubation time points. Data was acquired from a Bruker Q-TOF system using data dependent acquisition. Post-acquisition data processing was performed on a set of high resolution LC/MS/MS data files. The data, and its associated parent structure file, were automatically processed within the new software protocol.

Objective

The aim of the contribution was to evaluate the MetaSense™ software package in house on its suitability for our routine work-flow. Therefore, a metabolically well characterized development compound was incubated (s. below) and analyzed by LC-MS. The resulting 18 ion chromatograms were processed automatically by MetaSense™ and the results compared with the results we have obtained by routine data mining.

Material and Methods

Incubations: The compound was incubated with liver microsomes of rat, dog and man (Corning, Woburn, MA, USA) as follows: phosphate buffer (100 mM) with $MgCl_2$ (5 mM) were mixed with the test compound solution (25 µL, 10 µM) and microsome suspension (25 µL of a CYP content of 3 nmol/mL), the Incubation was initiated by addition of 50 µL cofactor after 5 min. of pre-incubation. Each time point (0, 5, 10, 20, 40, 60 min) of the incubation was stopped by addition of 250 µL acetonitrile/DMSO (9:1). The samples were then vortexed and centrifuged for 15 min at 18°C. The supernatant was transferred into the HPLC vial.

Analytics; Data were generated with a Dionex UltiMate® 3000 Rapid Separation LC (RSLC) UHPLC system with auto sampler and column oven (Thermo Fisher GmbH, Idstein, Germany) connected to a Maxxis impact TOF mass spectrometer (Bruker, Bremen, Germany). HPLC: column: ACE Excel 2 C18 150 x 2.1 mm; eluent A: 5 mM $(NH_4)HCO_2$ in H_2O (pH: 8.4); eluent B: 5 mM $(NH_4)HCO_2$ in MeOH, flow: 0.35 mL/min; step gradient over 36 min.

Results

In order to keep computing time in an acceptable range, the search was limited on 20 metabolites per run. The ones found, covered all previously identified pathways. The -in our opinion- most important result was the hit rate. Only view false positives (about 15% per species) were found. This seems to be mainly due to the peak-finding strategy: 1st metabolites are generated *in silico*, 2nd from the elemental formulae of the proposed metabolites, the accurate mono-isotopic $[M+H]^+$-masses are calculated, 3rd the masses are extracted from the TIC, 4th the peak shapes in the XIC are verified and finally 5th the found isotope patterns compared with the calculated patterns.

More metabolites could have been identified by changing a couple of the search criteria (e.g. noise level, max. number of hits, etc.) but would have required more powerful hardware than the present test system. Figure 1 shows the main screen (structures and masses could not be shown be legal reasons).

MetaSense™ proved its capacity to accelerate the work-flow of *in vitro* investigations and structure elucidation remarkably:

- The prediction of metabolites, whether from MetaSense™ itself or other software (e.g. Metasite) provides a 'mass list' so that the resulting MS/MS-experiments can be implemented in the sample sequence right from the beginning. In this case MetaSense™ showed to be able to predict all relevant metabolites correctly.
- Automated processing based on accurate mass combined with a comparison of the calculated and the detected isotope pattern data allowed a reliable identification of metabolites.
- Kinetic plots on parent decrease and metabolite increase are generated automatically (shown) and allow the calculation of Cl_{int}-values as well as metabolite formation rates simultaneously without operator intervention (scheduled).
- Biotransformation maps are created automatically for a given species for a certain time point or across different time points as a species specific one (shown). Needless to say that the same procedure can be applied to enzymes (e.g. CYP450) as well.
- Important data as unique human metabolites, species differences in the metabolic patterns can be displayed automatically (scheduled).
- Beside the automated data analysis, the option to add data obtained manually remains.
- The individual structures are connected to all relevant chromatograms, MS-, UV- and NMR-spectra, retention times, mono isotopic $[M+H]^+$-masses, calculated pK_s- and pK_b-values, IUPAC-names and meta data.
- Automatic compilation of study reports with all relevant information, relieves study director as well as technical staff from tedious, exhaustive, error-prone reporting.
- Progress of the investigation becomes transparent for everyone involved the process.

Conclusion and Outlook

As described above, MetaSense™ showed convincingly its “capacity saving” ability. Beyond these advantages, we believe that MetaSense™ integrates the Metabolism-work-packages excellently into the already existing ACD-landscape. It thus allows not only paperless communication, but permits also other departments and units access to potentially important data without demand, simply by accessing a common database. The same applies to a metabolism Lab: pKs and solubility data are crucial for in vitro experiments, development of HPLC- and HPLC-MS/MS methods. Impurities need to be known early, products from chemical degradation are often also formed by enzymatic activity, etc.

Points to consider: Powerful software like MetaSense™ require (i) skilled and experienced users, (ii) performant IT-support, (iii) standardized work-flows and finally (iv) a common understanding of the contributing units.

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Figure 1: Metabolic pathway (left), chromatographic and MS-data (center) and biotransformations found including their kinetic behavior (right).