Molecular Networking Approach combined with MetWork web server for Toxicology and TDM Application

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Background: In TDM/Toxicology, analysts are confronted with complex problems, resulting in potential important clinical consequences. Untargeted screening is a challenge, given the high number of molecules to be detected. LC-HRMS, the reference method for screening, generates a large volume of high quality spectral data, with a lack of tools for visualizing and organizing MS data. We applied molecular networking (MN) for untargeted screening interpretation. The objectives were to build a MS library and apply this database (DB) for drug's identification; compare theoretical MS libraries obtained *in-silico* with our DB to broaden its application; use MetWork webserver for metabolites identification by generating putative structures and predict the associated MS/MS spectra when the exact mass is detected in the network. **Methods:** For the clinical samples, plasma was crushed with acetonitrile. After centrifugation, the supernatant was injected onto an Ultimate 3000 LC-Orbitrap QExactive LC-HRMS system. For MS, positive MS and two data-dependent MS/MS scans of the two most intense ions were recorded. For MS/MS data, CID activation types was recorded. For MN, a data preprocessing using MZmine has been done prior to MN generation. The MN data were analyzed and visualized using Metgem. Finally, MetWork annotation, using an online tool (https://metwork.pharmacie.parisdescartes.fr), were filtered and this file was used to annotate the previously obtained MN. **Results:** For the DB, around 200 compounds were recorded including drugs found in hospitalized patients. Using this DB, we confirmed in more than 50 patients, among others, intake of bromazepam, amitryptiline, tramadol, voriconazole. The MN approaches confirmed results obtained by references methods used daily. Between both approaches around 80 % of the compounds were in common. MZmine data-preprocessing parameters were important to simplify the MN, reducing redundancy and therefore focus on real compounds. In a 2nd step, comparison was made between our experimental DB and *in-silico* MS/MS spectra using Competitive Fragmentation Modeling for metabolite IDentification to further annotate drugs metabolites. CFM-ID gave comparable results in 20% of cases. In a 3rd step, using MetWork web server, additional metabolite annotations were possible. Indeed, having one node identified in the MN, the server generates structures and, a similarity comparison between theoretical and experimental MS/MS spectra is performed to annotate the nodes. As an example, metabolites and parent drugs such as N-/O-Desmethyltramadol and tramadol, or carbamazepine/oxcarbamazepine were linked. **Conclusions:** Our results show that MN opens perspectives in TDM and toxicology using LC-HRMS. Combined with CFM-ID and MetWork to extend the annotation of new potential drugs or old drug's metabolites, even without reference standard, it could help clinicians to better understand drug-drug interaction and explain potential toxicity or lack of efficacy in patient's treatments.