An Infusion "Shotgun" Approach for High-Throughput Untargeted Metabolomics

Zuzana Demianova, SCIEX Germany

Introduction:
Metabolomics studies the complexity and large variety of various chemical compounds. Mass spectrometry-based untargeted metabolomics requires approaches to collect data in an unbiased fashion. Current LC approaches are biased to the column chemistry and metabolites which can be retained on such chemistries. The main goal of researchers in the untargeted metabolomics field is to analyze a large number of samples and obtain the most information in the shortest time with limited sample preparation. Here we evaluate the direct infusion or "shotgun" approach to such analyses by using a technique, Infusion MS/MSALL Acquisition, for untargeted metabolomics. This data-independent technique allows the MS/MS of all possible candidates, improve identifications significantly, allows to have quantitative data and enables retrospective analysis of the data.

Methods:
Data were acquired using a TripleTOF® 6600 System (SCIEX) coupled to a high flow liquid chromatography system (Shimadzu) and employing the Infusion MSMSALL Acquisition to collect one survey scan and then MS/MS of every single precursor within a given mass range. Mass ranges were evaluated from 50 to 900 in varied sizes to optimize the number of MS/MS and time needed to collect the data. Data were collected on a small case study of urine extract and statistical analyses was performed. Data were processed using MasterView™ Software and the accurate mass metabolomics spectral library for fast metabolite identification and confirmation. Statistical analysis was completed in MarkerView™ Software and any quantitation results were generated by MultiQuant™ Software.

Results:
By optimizing for mass range, MSMS ALL method was developed to capture all relevant metabolites per ionization mode (positive and negative mode). Metabolites are extracted from several biological samples and directly infused by using the developed method. The Total Ion chromatogram shows the presence of a large number of separate peaks. The extracted ion chromatograms show that most peaks represent, as expected, different metabolite classes, which are characteristic for each sample. Preliminary, by using the fragment filter tool, metabolites classes occurring in the different samples, were identified on the basis of their diagnostic fragment peaks and/or neutral loss. Statistical analysis is successively performed to differentiate samples and highlight rapidly marker compound in each sample. Using the high quality of MS/MS spectra thanks to SWATH acquisition, identification of marker and occurring metabolites is obtained together with the relative amount. "Shotgun" approach for Metabolomics results a powerful and rapid tool to obtain huge and complementary data of numerous samples even if metabolites present only at very low levels in biological samples. Post-acquisition data processing software, allows the user to have rapidly structural information and/or identification and quantification of metabolites occurring in complex samples to derive important biological conclusions.
By using Infusion MSMSALL with SWATH acquisition, a rapid metabolic fingerprint of each biological sample can be captured. The optimized method is able to capture all relevant metabolites per ionization mode (positive and draft 391 negative mode). Verifying the method through a simple biological extract case study highlighted the metabolites responsible for differentiating the different sample groups.