Construction and application of a high-resolution MS/MS library including retention time information for rapid identification of endogenous metabolites

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1. Introduction

High-resolution LC-MS is an important platform for metabolite detection and quantitation. However, for untargeted Metabolomics rapid, unambiguous and universal compound identification is still challenging. In this work, we report the construction of a library for relevant endogenous metabolite and its' successful application to human biofluids.

2. Approach

Standards were obtained from the Human Metabolome Database (HMDB). They were injected into an Intensity Solo C18 column via an Elute LC system and detected by a QTOF-MS (impact II, Bruker Daltonics) for acquiring MS/MS library spectra and the retention time determination (RT).

For the analysis of biofluids the same setup was used following a standard operating protocol (SOP) for consistency. Automatic metabolite identification was performed in the MetaboScape software based on matching of multiple parameters: precursor mass accuracy and isotopic pattern, RT, and MS/MS spectrum quality.

3. Results

In this study a library from over 800 endogenous metabolites was created. It contains MS/MS spectra of 635 compounds acquired in positive mode and 474 negative mode spectra. Up to 5 collision energy levels were applied for each standard giving more than 6000 MS/MS spectra in total. For each metabolite, library fragment spectra were manually curated by confirming each fragment via a molecular formula. For unambiguous identification we determined the RT of each standard. An RT correction method using RT standards was applied to balance effects caused by variations in experimental conditions.

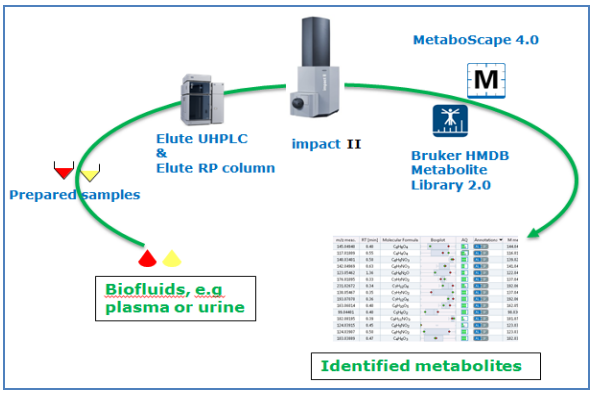

Figure 1. Non-targeted metabolomics workflow solution: from unprepared biofluid samples to identified metabolites.orem ipsum.

Finally, we have examined the portability of this library for different labs. Biofluids such as human urine were analyzed in both positive and negative ion modes in LC-MS, followed by metabolite identification using the library. The comparative results will be presented.

4. Discussion

A complete solution for non-targeted Metabolomics based on UHPLC QTOF was developed:

- for a portable, universal, reliable and simple workflow
- including SOPs for clinical samples, data acquisition and evaluation
- for providing a head start in non-targeted metabolomics research