SECIM NMR Workflow for Metabolite Profiling of Biofluids

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Introduction

Metabolomics is an emerging field conveying applications to bioscience and medicine. The Southeast Center for Integrated Metabolomics (SECIM) has been funded by the National Institutes of Health (NIH) Common Fund's Metabolomics program to provide services to users in all areas of biomedical and biological sciences. Herein, we show the established workflow for biofluids in the NMR core.

Experimental

In general samples are kept at -80°C until sample preparation. When ready for analysis, aliquots are mixed with a buffer that is appropriate for the sample type. A Gilson 215 Liquid Handler with Gilson Trilution 2.0 software is used to automatically prepare samples. NMR data are collected on a Bruker Advance II 600 MHz spectrometer using a 5 mm cryoprobe, equipped with a SampleJet sample changer allowing storage at 6 °C and automatic measurement in the AMRIS facility at the University of Florida.

Results and Discussion

Several research groups have published different methods and measurements for NMR based metabolomics.[1,2,3] Figure 1 shows the SECIM workflow for biological samples. In each set of samples, buffer and quality control samples are added to identify any cross contamination and quality control of overall procedure. Data are processed in NMRPipe and analyzed in MATLAB. In addition to 1D NMR, 2D ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC), total correlated spectroscopy (TOCSY) and 2D homonuclear J-resolved spectra are collected on representative samples for the identification of metabolites. Metabolites are identified using complex mixture analysis by NMR (COLMAR) ^{1}H-^{13}C HSQC query[4] and validated by TOCSY overlays of reference spectra from the Biological Magnetic Resonance Data Bank (BMRB) as well as Statistical total correlation spectroscopy (STOCSY)[5]. We are currently developing protocols to apply Bayesian automated metabolite analyzer for NMR (BATMAN)[6] to 1D NMR data for quantification following metabolite ID using COLMAR.

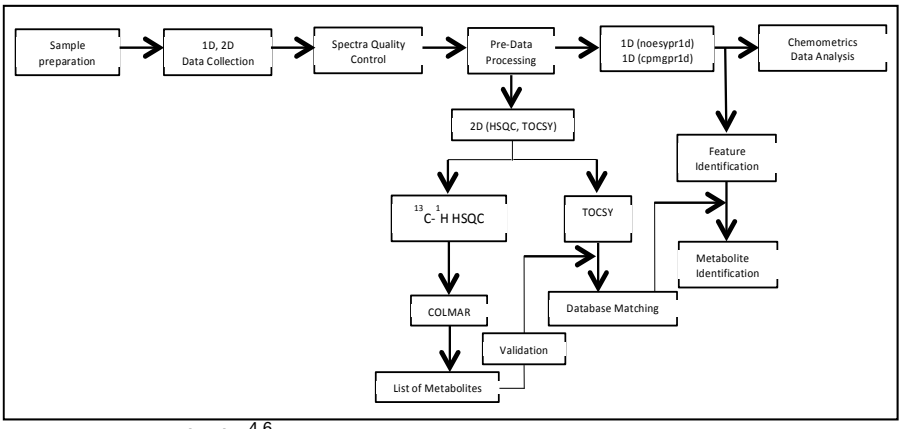

Fig.1 Overview of SECIM experimental workflow

Conclusions

SECIM NMR core offers services include, sample preparation, NMR analysis and assistance in data processing and analysis. The aim of this project is to establish an efficient and robust workflow for metabolite identification and quantification that can be applied to a wide range of samples from biofluids to plant extracts using COLMAR ^{1}H-^{13}C HSQC query and Batman, respectively.[4,6]

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