Metabolite Profiling of NSAID Effects In Vitro Using Shimadzu LCMS-IT-TOF System With Phenomenome Profiler™ Software

Yasuyo Yamazaki[1], Yusuke Inohana[2], Shinichi Yamaguchi[2].
[1]Phenomenome Discoveries Inc., Saskatoon, SK, Canada, [2]Shimadzu Corporation, Kyoto, Japan

Overview

Global metabolite profiling software was used to measure and compare the metabolic changes induced by several NSAIDs in HT29 cells and media. This approach can be used as a model system in monitoring drug metabolism in vitro.

To help find components of biological significance the Shimadzu LCMS-IT-TOF was used to provide high mass accuracy MS^n data integrated with Phenomenome Profiler™ software to integrate chromatography and mass spectrometry data sets together with statistical methods.

Phenomenome Profiler™ software is the world's first universal metabolomics software solution developed specifically for metabolomics research projects.

It is an integrated suite of algorithms, statistical methods, and computer applications to support global profiling of complex mixtures using MS.

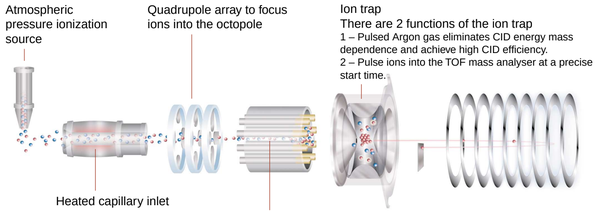
Shimadzu Corporation are a key partner with Phenomenome Informatics, a division of Phenomenome Discoveries

Introduction

Human cancer cell lines are invaluable tools for many aspects of basic cancer research, ranging from purely investigational studies to drug efficacy and toxicity studies. The ability to measure endogenous and excreted metabolites over the course of such studies creates an opportunity to quantify the resulting phenotypes. Metabolites found to correlate with specific biological events are highly informative, and can be utilized as biomarkers for numerous applications, including disease diagnosis, drug efficacy and toxicity, as well as basic investigational research. Traditionally, the most common platforms for studying metabolites have included LC-MS, GC-MS and NMR. Recent technological advancements in MS instrumentation, such as the Shimadzu LCMS-IT-TOF system, enable the acquisition of high resolution and high mass accuracy MS data suitable for novel metabolite discovery. Although these instruments have been developed, no complementary software has been designed specifically to interpret resulting metabolomics data. To address these issues, we have developed Phenomenome Profiler™ software to handle MS spectrometry-based analysis of biological samples. Phenomenome Profiler™ also easily performs a number of multivariate statistical functions on the raw data.

Methods

HT29 human colon cancer cell lines were grown to 80% confluency, and were treated with four drugs using high and low doses (listed below). After 24-hour treatment, media from each triplicate treatment was analyzed randomly. A total of 27 samples were processed via a proprietary extraction method, optimized to separate the metabolites into multiple extracts based on polarity and acid/base chemistry. HPLC was carried out on an LC-10AD*VP* pump and SIL-10AD*VP* auto-injector. MS analysis was performed on the LCMS-IT-TOF using an ESI source. All instruments and columns described above were obtained from Shimadzu Corporation (Kyoto, Japan).



Name	Drug	Dose	Name	Drug	Dose
ASA - HD	ASA (aspirin)	high - 3mM	Indo - HD	Indomethacin	high - 800µM
ASA - LD	ASA	low - 1mM	Indo - LD	Indomethacin	low - 100µM
Cel - LD	Celebrex	high - 100µM	Viox - LD	Vioxx	high - 200µM
Cel - LD	Celebrex	low - 10µM	Viox - LD	Vioxx	low - 20µM
DMSO	none (solvent)	none			

Results

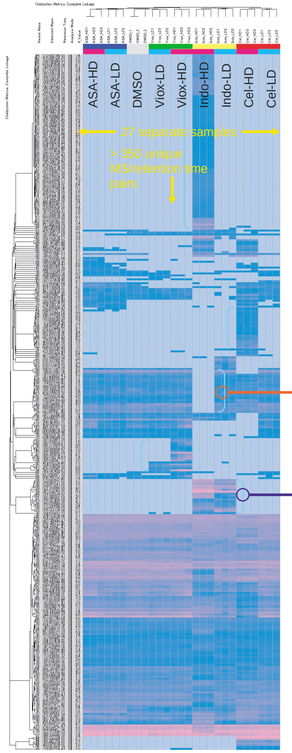


Figure 2. Metabolite array of drug-treated HT29 human cancer cell line samples.

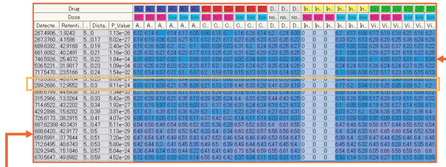


Figure 3a. Indomethacin-related down-regulated endogenous metabolites;

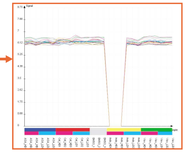


Figure 3b. Metabolite coordinate plot.

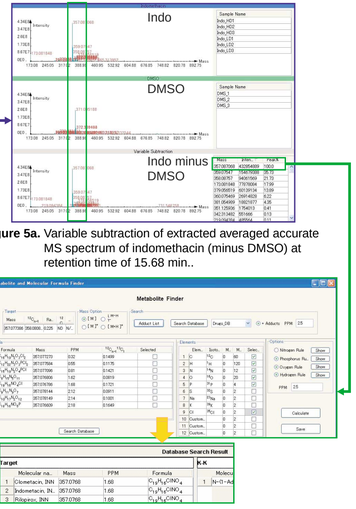


Figure 5a. Variable subtraction of extracted averaged accurate MS spectrum of indomethacin (minus DMSO) at retention time of 15.68 min..

Figure 5b. Molecular Formula Finder assigns the molecular formulae based on accurate MS.

In total, 27 drug-treated HT29 cancer cell line samples were analyzed on the LCMS-IT-TOF system using a positive ion ESI with a scan range of 150 to 1000 m/z. All unique MS/retention time pairs represent sample-specific metabolites, and metabolite fragments are visualized in a metabolite array (Figure 2).

Purple represents metabolites with the greater intensity and followed by dark blue, and light blue represents undetected metabolites. Each column of the array represents one sample, and each row represents a single MS/retention time pair.

The array can then be analyzed, and key metabolites identified, using numerous statistical (unsupervised analysis) and Boolean search(supervised analysis) tools incorporated in the Phenomenome Profiler™ software.

As displayed in Figure 2, the array has been clustered hierarchically by sample and metabolite, as indicated by the dendrogram at the top and left of the array. As you can see clearly by the array, drug-related metabolites are clustered together.

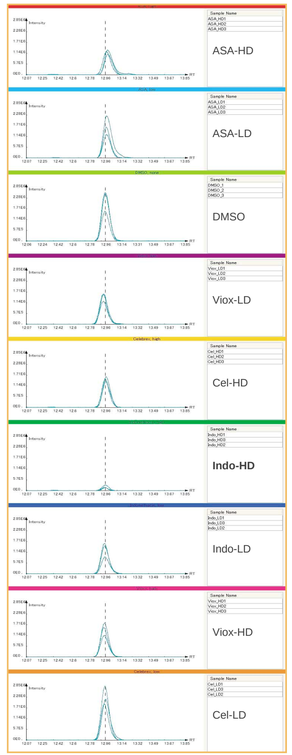


Figure 4. Extracted accurate MS ion chromatogram at m/z of 289.269.

Discussion and Conclusions

For the comprehensive metabolite analysis, we employed a Shimadzu LCMS-IT-TOF (Figure 1), a new hybrid MS spectrometer combining an ion trap with time-of-flight. This instrument enables the acquisition of MS and MS^n spectral data featuring high resolution, high mass accuracy, and high sensitivity. These valuable attributes are due to the following features of the LCMS-IT-TOF: compressed ion introduction methods, improvements to Dual Stage Reflection, ion cooling using argon gas, and a temperature-controlled time of flight mass analyser.

Phenomenome Profiler™'s new profile-matching tool uses the same concepts as other cluster analysis tools used in multivariate statistical analyses. By simply selecting the metabolite of interest from the array, this tool will extract all other metabolites with the same expression profile. As pictured in Figure 3a, by selecting the indomethacin-high dose (indo-HD) effect, the program indicates any indo-HD-related down-regulated endogenous metabolites. Phenomenome Profiler™ will also display the expression pattern of those selected metabolites, as shown in Figure 3b.

Real-time interaction exists between the array tables and the underlying raw chromatogram and MS spectrum data. Figure 4 shows the extracted accurate MS ion chromatogram (EIC) of the indicated row from Figure 3a. In this figure, the assigned variables are grouped together and displayed as an EIC overlay.

Some of the metabolites of interest were related to indomethacin metabolism in vitro; these were extracted from the array (Figure 2), and are displayed in Figure 5a as a variable subtraction of the extracted MS spectrum. According to the accurate mass information, we can easily assign the putative molecular formula—one of the remarkable features of Profiler™ software—as shown in Figure 5b. In this example, the detected metabolite was indomethacin itself. The Molecular Formula Finder also identifies carbon-13 metabolites in the same array.