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WP 549

Insight Profiler streamlines analysis of non-targeted metabolomics data from LCMS or direct injection in a single software solution

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Overview

- Application of Insight profiler, a novel software solution for non-targeted metabolomics analysis, to reveal potential candidate biomarkers for pancreatic cancer research.
- Insight Profiler automates complex data processing bringing together feature detection and alignment, statistical analysis, filtering and compound identification into a single processing method cascade applicable to LC-MS/MS and direction injection HRMS.
- Statistically significant features identified by LC-MS/MS were also significant in direction injection analysis, highlighting its potential as a future screening workflow.

1. Introduction

Advances in non-targeted metabolomics increasingly rely on integrated workflows which are capable of handling complex spectral deconvolution, feature alignment, and multivariate interpretation. Several applications provide complementary capabilities for ion feature extraction and statistical interrogation; however, transitioning data across discrete platforms increases workflow fragmentation when working with large scale metabolomics studies. In this work a single software application has been developed that can automate feature detection and alignment, statistical analysis and filtering with spectral annotation delivering an integrated solution within a single processing method. The data processing application has been applied to a biomarker discovery study with pancreatic cancer serum samples from donors and controls analyzed by LC-MS/MS and direct injection MS on a QTOF.

2. Materials and Methods

Serum samples included healthy controls (n=30) and pancreatic ductal adenocarcinoma (PDAC) (n=30). The research project was approved by the Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) Tissue Access Committee. Serum extracts were profiled using high resolution direct probe ionization MS (DPiMS) as well as reversed-phase LC-MS/MS (LCMS-9030 Q-TOF system, Shimadzu Corporation, Japan).

All data were processed and analyzed in a novel software solution for non-targeted analysis (Insight Profiler, Shimadzu Corporation). Data processing in Insight Profiler software considered feature detection, alignment, statistical analysis and compound identification in a single method. Compound identification used an in-house metabolomics library, as well as third-party repositories including MassBank, LipidBlast and HMDB.

2.1 Data acquisition

Reversed phase LC Separation.

- C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min
- Binary gradient; water + 0.1% formic acid and acetonitrile + 0.1% formic acid.
- Sample cycle time 35 minutes.

LC-MS/MS Mass Spectrometry Detection.

- Positive ion mode TOF MS survey scan (m/z 60-1000; 100 msecs)
- 27 DIA-MS/MS mass scans (m/z 40-1000; 33msec; precursor isolation 35 Da)
- Collision energy spread 5-55V; External mass calibration. Total cycle time <1 sec.

DPiMS Mass Spectrometry Detection.

- TOF MS mass scan m/z 100-1500; 2 min analysis, polarity switching.

2.1 Automated data processing in Insight Profiler

Insight Profiler software has been developed to automate complex data processing in non-targeted analysis applications including metabolomics under a single processing method cascade.

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak.

Statistics and filters
Applied to find ion signals of significance and to remove ions of high variance.

Compound identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.

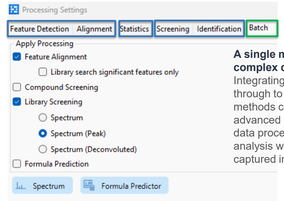

Figure 1. The Insight Profiler processing application method editor, designed to create a single workflow for feature detection of unknowns to compound identification.

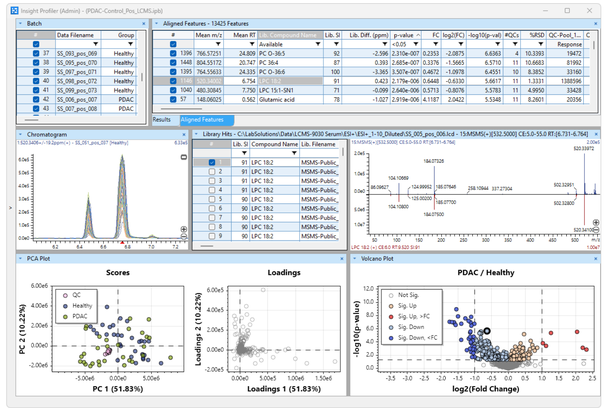

Figure 2. Insight Profiler was applied to the analysis serum extracts from PDAC patients and healthy controls following non-targeted metabolomics analysis (LC-DIA-MS/MS). Interactive and intuitive data review enables analysis of statistically significant features. Chromatogram, spectrum and library identification are linked with statistical analysis, including PCA/Volcano plots, helping data interpretation to find potential biomarkers. The same software was applied to analysis of the DPiMS dataset with simultaneous analysis of positive and negative ion features.

3.2 Potential PDAC biomarkers detected by LC-MS/MS

The software application was applied to a biomarker discovery study with pancreatic cancer samples analyzed by both HR LC-MS/MS and HR DPiMS (direct injection and no LC separation). Insight Profiler software analysis of LC-MS/MS data identified significant differences in metabolite profiles compared to healthy serum controls. Consistent with previously published HR LC-MS/MS literature, phospholipids containing linoleic acid were significantly reduced (for example LPC 18:2, PC 18:1_18:2, PC 18:2_18:2 and PC 18:2_20:4, were putatively identified by MS/MS as shown in Fig. 4). LPE 18:2 was also reduced alongside LPC 18:1, LPC 18:3 and LPC 20:5. Glutamic acid was significantly higher in PDAC serum profiles compared to controls.

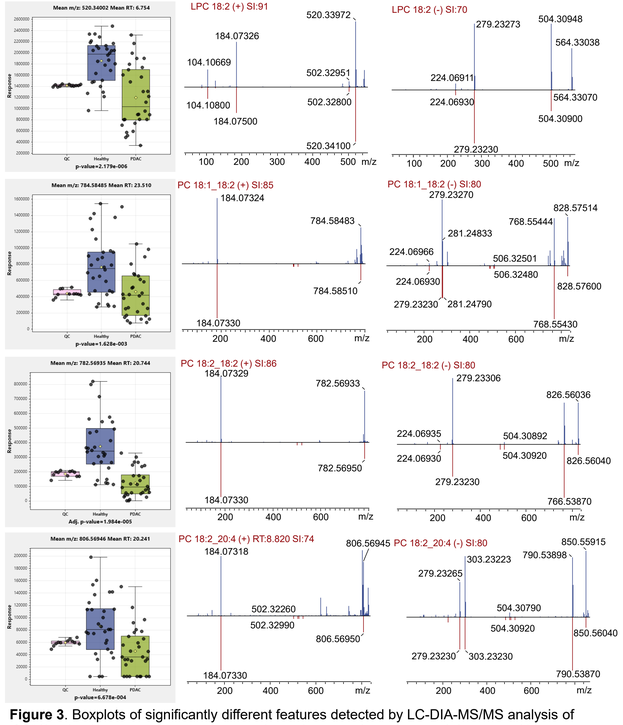

Figure 3. Boxplots of significantly different features detected by LC-DIA-MS/MS analysis of PDAC patient serum samples compared to controls. Features were putatively identified in positive and negative ion mode data to provide higher confidence in MS/MS identification.

3.3 DPiMS screening for detection of biomarkers

Direct Probe Ionization Mass Spectrometry (DPiMS) is a rapid, minimal-sample-preparation ambient ionization technique that enables high-throughput profiling of complex samples by direct analysis of solids or residues without chromatographic separation. Insight Profiler data processing software was applied to DPiMS/QTOF using the same default setting as in LC/QTOF analysis highlighting the possibility of applying DPiMS in a broader context for biomarker screening studies.

Figure 4. Insight Profiler was applied to the analysis serum extracts from PDAC patients and healthy controls following non-targeted direct injection analysis (DPiMS). The software automatically extracts positive and negative ion features for statistical analysis. In the example shown, the ion signal m/z 542.32218 detected in positive ion mode was statistically significantly lower in PDAC samples compared to controls, corresponding to LPC 18:2 identified by LC-MS/MS. Consistent with LC-MS/MS, LPE 18:2, LPC 18:1, LPC 18:3 and LPC 20:5 were also significantly lower in PDAC samples; glutamic acid was significantly increased.

4. Conclusions

- A single software application developed to automate feature detection and alignment, statistical analysis and filtering with spectral annotation was applied to a biomarker discovery study with pancreatic cancer serum samples from patients and controls analyzed by direct injection MS and LC-MS/MS on a QTOF.
- Using the same Profiler software, a processing method applied to HR DPiMS data showed correspondence between the LC-MS/MS and DPiMS techniques and highlighted metabolite features that may be considered for unique screening techniques for PDAC research.
- Using a single processing method cascade to automate metabolomic data analysis helps to simplify complex data processing workflows without compromising data quality and highlights the possibility of applying DPiMS within broader metabolomics research projects.