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Combining Chromatographic Deconvolution with Electron Ionization and Chemical Ionization for Unknown Identification with High-Resolution Accurate Mass GC/MS

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ABSTRACT

Purpose: Evaluate the use of Compound Discoverer 3.2 software for processing GC-Orbitrap data.

Methods: Data was acquired from different oregano samples and processed using Compound Discoverer's electron ionization (EI) and chemical ionization (CI) processing nodes.

Results: Good PCA separation was seen with EI data. Compounds could be identified in both EI and CI that contribute to sample variation, indicating a likely adulteration of one of the samples.

INTRODUCTION

Chromatographic deconvolution has been used in untargeted GC/MS software to group individual ions together into a compound spectrum. In electron ionization (EI) workflow, identification is typically accomplished by searching against spectral libraries. Chemical ionization (CI) is essential in structural elucidation of tentatively identified compounds or true unknowns because it allows for more confident molecular ion identification. With the growing popularity of high-resolution accurate mass GC/MS, there has been growing interest to process both EI and CI data in one GC/MS software to improve the confidence of unknown compound identification. In this study, a software platform which integrates classical GC/MS EI and CI workflows will be presented using data acquired from different oregano sample types on a GC-Orbitrap mass spectrometer.

METHODS and RESULTS

Thermo Scientific™ Compound Discoverer™ 3.2 software was used to process data acquired on a Thermo Scientific™ Q Exactive™ GC mass spectrometer. Compound Discoverer is a node based high resolution accurate mass data processing software where the processing workflow can be configured by the user to fit the end goal of the experiment.

Electron Ionization workflow

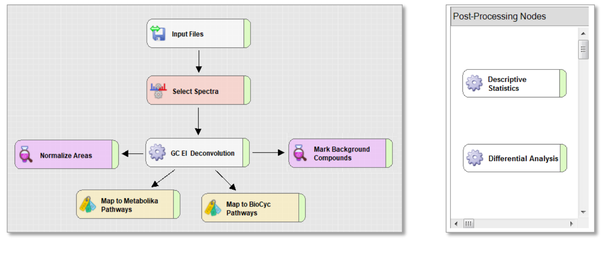
The first workflow configured for this experiment used the GC EI Deconvolution node as the core processing node. This node can perform chromatographic peak deconvolution, retention indexing, identification by spectral library search and cross sample peak grouping (Figure 1).

Figure 1. GC EI Deconvolution node functionality

Figure 2 below shows additional nodes that would be connected to the GC EI Deconvolution node in a typical processing workflow. They are summarized as follows:

- Normalize Areas – Normalizes area drift across the batch
- Mark Background Compounds – Flags compounds in samples that are also present in blanks
- Map to metabolomics pathways – Highlights identified compounds in metabolomics pathways
- Statistics post processing nodes – Provides data and charts related to statistical analysis

Figure 2. GC EI Deconvolution node connected to other nodes in Compound Discoverer



If statistics post processing nodes are selected, replicate samples can be grouped according to sample type. In the example shown in Figure 3, two types of oregano samples were analyzed (2980 and 7875), and these oregano types were pooled together to create a third type (Blend). Three sample extractions from each oregano type were grouped together, creating three sample groups.

Figure 3. Three types of oregano samples are added to three sample groups

Sample Identifier	Sample Type	Oregano Type
7_2980C(1)	Sample	2980
8_7875Z(1)	Sample	7875
9_Blend(1)	Sample	Blend
10_Blend(2)	Sample	Blend
11_7875Z(2)	Sample	7875
12_2980C(2)	Sample	2980
13_7875Z(3)	Sample	7875
14_Blend(3)	Sample	Blend
15_2980C(3)	Sample	2980
44_Oregano_PCI	Sample	n/a

Figure 4 below shows the resulting PCA plot generated in the data review pane after processing the GC EI Deconvolution node with the Descriptive Statistics and Differential Analysis post processing nodes. Good separation is seen between sample groups indicating the samples have distinct chemical compositions when compared to each other.

Figure 4. PCA plot generated in Compound Discoverer showing good group separation

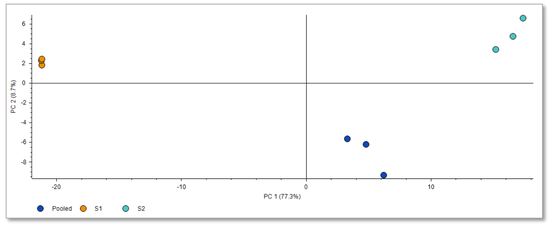
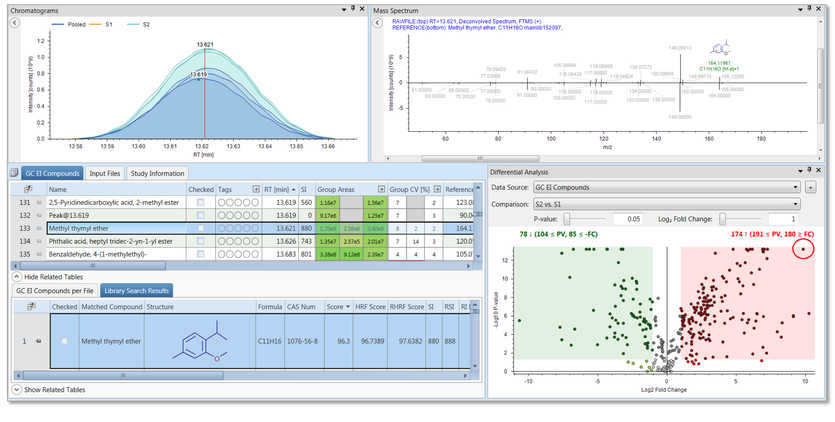


Figure 5 below shows the full data review pane for the processed batch. The volcano plot gives a comparison between the two sample groups before pooling. The upper corners of the volcano plot show compounds that provide the biggest contribution to the variation between the two sample types. Clicking on the data point circled in red in the upper right hand corner of the plot highlights this compound in the compound table, displays an overlay of its extracted ion chromatogram across the batch, and presents a deconvolved spectrum together with its top library hit. This compound is identified as methyl thymyl ether. Given that this compound is not frequently found in oregano, its high concentration in the sample indicates the sample has likely been adulterated or otherwise contaminated.

Figure 5. Data review pane showing volcano plot and identification of methyl thymyl ether



Chemical Ionization workflow

Similar to the EI workflow, the GC CI Deconvolution Node can also perform chromatographic deconvolution, retention indexing, library search and cross sample peak grouping. Figure 6 shows a typical CI workflow as it would be set up in Compound Discoverer.

Figure 6. Typical CI workflow in Compound Discoverer

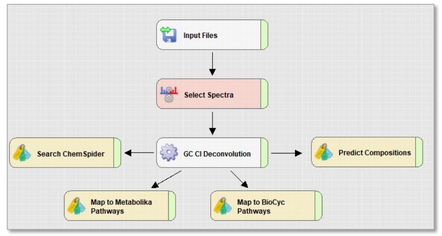
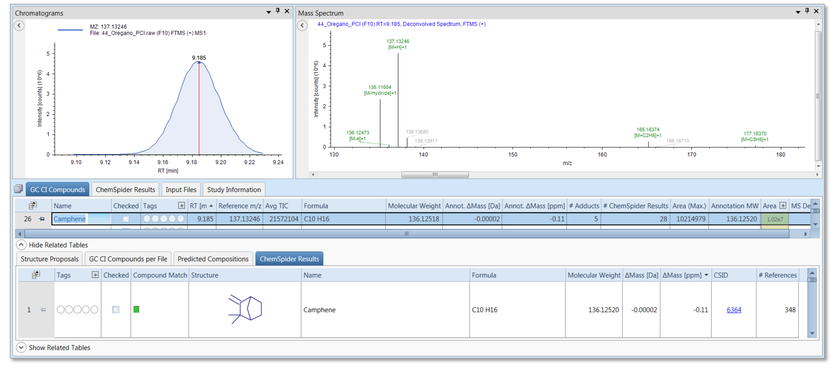


Figure 7 below shows the data review pane after processing CI data. Featured in the upper right corner is the molecular ion adduct pattern of the selected compound. In this case methane was used as the CI gas, so $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ adducts were found and annotated in the spectrum, helping to confirm the molecular weight. The formula was determined from the predict composition node, and this formula was automatically searched against ChemSpider databases to suggest possible structures. Camphene was selected as a tentative identification from this list. This workflow is useful to assist in identification of compounds when no EI reference spectrum is available.

Figure 7. Data review pane for CI workflow



CONCLUSIONS

- Compound Discoverer 3.2 proved useful in analyzing variations in oregano samples.
- Good PCA separation was seen over three oregano sample types, indicating variation between the types. Volcano plots helped highlight compounds that greatly contributed to this variation.
- EI library search allowed for identification of these compounds, and a CI workflow was demonstrated to be useful when no EI library spectrum is available

TRADEMARKS/LICENSING

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