Automatic detection and grouping of MS^1 fragmentation in LC-MS data

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Abstract

Purpose: Optimize data acquisition to enable reduced complexity in LC-MS data by the automatic detection of MS^1 fragments during untargeted component detection.

Methods: Data was acquired using two different approaches to assess the ability to improve detection and grouping of MS^1 fragments. Firstly, a common set of conditions using 30, 50, and 80% NCE.

Results: Optimized data acquisition conditions provided an increase if detection and grouping of MS^1 fragments by 55% in positive ESI ionization LC-MS data and by 16% in negative ESI LC-MS data.

Introduction

Analysis of complex samples by LC-MS is made more difficult by the formation of fragments in MS^1 level data formed during the ionization and transmission process. These MS^1 fragments represent additional complexity combined with isotopes and adducts that need to be detected and grouped to create a correct analysis of compounds. Unlike isotopes and adducts which can be more easily determined using known mass deltas, MS^1 fragments are more difficult to determine. Utilizing a combination of online spectral libraries with very low energy data and optimized data acquisition, we present a method for detection and grouping of MS^1 fragments.

Materials and methods

Sample preparation

A dried sample of milled ribwort plantain leaves was reconstituted with 2 mL water and well mixed. After mixing, 3 mL methanol was added, and the sample was well mixed. The sample was centrifuged to separate insoluble components with the supernatant filtered through 0.3 µm filter. The sample was evaporated to dryness and reconstituted in 160 µL methanol and 840 µL water for the final sample.

Test method(s)

Samples were analyzed by LC-MS on a Thermo Scientific™ Orbitrap™ ID-X™ Tribrid™ mass spectrometer connected to a Thermo Scientific™ Vanquish™ Horizon LC system. Separation was achieved on a Thermo Scientific™ Accucore™ phenyl-hexyl column (100X2.1, 2.6 µm with mobile phase A consisting of water with 0.1% formic acid and mobile phase B consisting of ACN:MeOH with 10mM ammonium formate and 0.1% formic acid (47.5 : 47.5 : 5).

Time	%A	%B
0.00	84	16
1.00	84	16
14.50	20	80
14.51	1	99
15.50	1	99
15.51	84	16

Data analysis

Data analysis was performed using Thermo Scientific™ Compound Discoverer™ 3.4 software using an untargeted data analysis workflow with the detection of MS^1 fragments set to utilize both online library and internal MS^2 spectra for unknowns.

Results

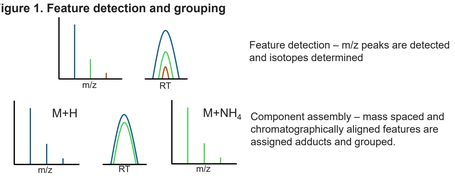
Detection and grouping of MS^1 fragment ions

During ionization and initial movement of analytes into the high vacuum area of a mass spectrometer, ions may undergo fragmentation. These MS^1 fragment ions increase the complexity of the data, producing false positive peaks during untargeted peak detection. A means to detect and properly group these MS^1 fragment ions along with the unfragmented adduct species would reduce this complexity and simplify interpretation.

While it is possible to look for co-eluting MS^1 signals and attempt to group them based on peak shape and retention time, this method can struggle to detect and group peaks with very different intensities. In addition, this method does not confirm that the grouped MS^1 ions are actual fragments of heavier MS^1 features instead relying only on co-elution. An alternative method utilizing fragmentation data could bolster confidence in detection and grouping. To obtain this fragmentation data could be taken either from an external fragmentation spectral library or from within the data set itself.

In short, the software performed an untargeted component detection but with the added step of considering and looking for potential MS^1 fragment ions. The process for component detection starts by detecting full scan features and assembling isotope groups, (Figure 1.) during this process a peak quality score was applied to remove signals with a quality score below 2 (of 10).

Figure 1. Feature detection and grouping

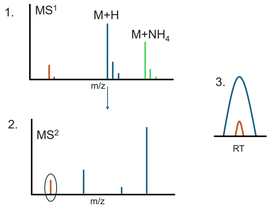


Feature detection – m/z peaks are detected and isotopes determined

Component assembly – mass spaced and chromatographically aligned features are assigned adducts and grouped.

In the next step, the detected isotope groups were assembled into compounds by identifying and grouping ionization adducts. It was during this step that detection and grouping of MS^1 fragment ions was also applied. For each component, the MS^2 spectra available were searched against a library (mzCloud™ software) which contained a wide energy range of fragmentation data including very low energy data on all compounds (10% NCE HCD up to 200% NCE HCD). If a hit for a component was found, the library also returned fragment ions observed for the putative candidate at low HCD energies. The software then searched the detected MS^1 components for those m/z values and if found to be present, and chromatographically aligned with another detected feature group, would be marked as an MS^1 fragment ion and assembled into the appropriate compound.

Figure 2. Detection of potential MS^1 fragments



1. Detected feature groups with MS^2 spectra or library ID are investigated.
2. Fragments in MS^2 spectra are used to interrogate MS^1 detected features.
3. Aligned feature peaks are grouped as MS^1 fragments into adduct grouped components.

Data acquisition for MS^1 fragment grouping

Normal settings for fragmentation acquisition focus on acquiring data sufficient to help identify compounds. Stepped or ramped fragmentation is a useful approach allowing the acquisition of a fragment spectra across a range of fragment energies. Typical values for positive ionization HCD fragmentation fall around 50% normalized collision energy (NCE) with stepped conditions such as 30, 50, and 80% stepped NCE being normal. While these higher energies can assure getting a suitable fragment spectra, they may not contain signal for MS^1 fragments that are generated at conditions similar to lower collision energies. For this work, we also acquired data using 10, 50, and 80% stepped HCD NCE to compare both for identification and potential improvement of MS^1 fragment grouping.

Figure 3. Full MS^1 (+) at 15.32 min. for the detection of peak 535.4825 showing the signal for 388.3204.

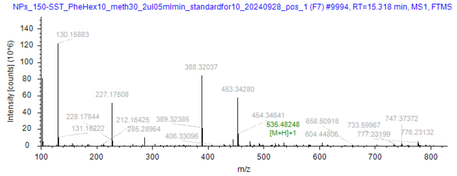


Figure 4. HCD MS^2 fragment spectra of 535.4825 using standard conditions (30, 50, 80%)

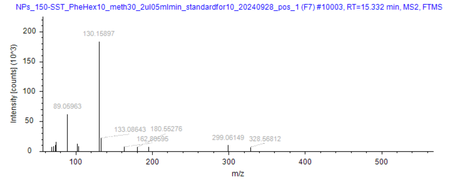
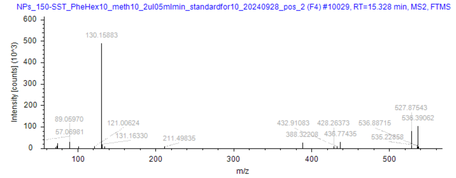


Figure 5. HCD MS^2 fragment spectra of 535.4825 using MS^1 fragment detection conditions (10, 50, 80%) showing signal for higher m/z ions including 388.3221



The lower energy acquisition was able to generate fragment ions in MS^2 spectra that were not present in the traditional 30, 50, 80% NCE HCD scans. These lower energy fragments allowed the software to detect MS^1 fragment ions in compounds that were not identified by a library search and could not be identified by the MS^2 spectra of higher energy scans. As an example, a component detected as m/z 535.4825 at RT 15.3 was correctly grouped with the MS^1 fragment ion 388.3203 only when the low energy spectra was available. (Figure 3, 4, and 5). The utilization of a specific low energy stepped energy scan was able to identify MS^1 fragment ions and group them successfully which led to an increase in MS^1 fragment and detection of 55% in positive ionization and 16% in negative ionization.

Impact of data acquisition on library ID

The inclusion of a low energy in the stepped conditions, intended to assist in detection and grouping of MS^1 fragment ions, may have an impact on the ability of the acquired stepped NCE scan to provide a good spectral library hit. To assess the potential impact, the ability to provide library hits based on the same sample with different fragment energies was also assessed. Overall, the impact of the inclusion of a low energy stepped collision energy was minimal. The total number of compounds returning a library hit with a score of above a minimum of 60 (of 100) was the same regardless of the acquisition in positive mode. When considering high quality library hits, the level of scores for potential IDs was marginally higher with the lower energy acquisition (Table 1) vs. lower energy for hits with a score above 80 of 100 using the Cosine scoring algorithm. The impact was similar in negative mode acquisition where the low energy acquisition resulted in a similar number of library hits and a higher number of higher score hits (above 80) for the lower energy acquisition.

Table 1. Library hits with a match score above 80 under different acquisition conditions

Polarity	(10, 50, 80% NCE HCD)	(30, 50, 80% NCE HCD)
Positive	217	195
Negative	129	103

The level of higher confidence hits, potential identifications that could be used in the detection of MS^1 fragments, across both positive and negative data provided a small improvement in MS^1 fragment detection however the more important aspect being that the inclusion of a low energy step energy to bolster MS^1 fragment detection and grouping for compounds without a library hit did not negatively effect library hits and potential IDs for this data set.

Future directions

The ability to detect and group MS^1 fragment ions through library spectra or acquired unknown data helped to reduce data complexity. While the use of specific low collision energy did increase the ability of the software to detect and groups MS^1 fragment ions and did not have a negative effect in the specific plant sample used in this study, the need to include a low energy stepped scan may still cause a negative effect on library hits for other samples or chemotypes. It may be possible to avoid this trade-off by specifically acquiring low energy MS^2 data in a separate injection for use in the data processing workflow allowing the ID MS^2 data to be acquired without the need for low energy.

Conclusions

The detection and grouping of MS^1 fragment ions utilizing

- Reference spectral libraries containing low energy data can be useful for providing expected MS^1 fragment ions.
- Inclusion of low energy acquisition can improve the detection of MS^1 fragment ions by increasing observable fragment ions in MS^2 spectra.

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