Identification of Compound Classes Using the Filter Results by Fragments Feature of Agilent MassHunter Software

Technical Overview

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Introduction

Identification of unknown compounds in complex mixtures is always a challenging task. Tools that can simplify the data would be highly useful to researchers dealing with complex biological samples. The Filter results by fragments feature in Agilent MassHunter Qualitative Analysis software is one such example of data simplification. It helps to filter the data based on the fragment mass or neutral loss of interest. Some lipids and metabolites form fragment ions that are diagnostic of a particular class of compounds[1]. For example, phosphatidylethanolamines (PE) show a neutral loss of 141 Da in their MS/MS spectra in positive mode. Prostaglandins show a neutral loss of 100 Da. Ceramides show a characteristic fragment ion at *m/z* 264, and phosphocholine-containing lipids show a diagnostic peak at *m/z* 184. Using the Filter results by fragments option, the presence or absence of prostaglandins/PE or any similar compound class can be identified more quickly than the conventional way of compound search across the chromatogram.

This Technical Overview describes a process to filter the data using the Filter results by fragments feature in the MassHunter software to identify ceramides, PEs, and phosphatidylcholines (PCs) in a cellular lipid extract.

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Filtering the data by fragment ion

Ceramides are a subclass of lipids. These lipids have an unsaturated C18 hydrophobic chain as a basic skeleton in the structure. A variable N-acyl amino chain is associated with this basic skeleton. The length of the N-acyl amino chain may vary from C14 to C24. In MS/MS, they show a characteristic ion at *m/z* 264, corresponding to the basic skeleton. It has been found that some ceramides have a saturated basic skeleton and are 2 Da heavier than the unsaturated species. MS/MS spectra of these ceramides show a characteristic ion at *m/z* 266.

Filtering the data using these two diagnostic ions will be helpful during the analysis of a complex mixture containing different kinds of ceramides. The tool, Filter results by fragments, can generate two different lists for the two types of ceramides.

We extracted lipids from THP-1 cells. The total extract was analyzed by LC-MS/MS in an untargeted data-dependent manner. We observed some MS/MS spectra containing a dominant ion at *m/z* 264.269, followed by a low abundant 282.2785 ion (Figure 2A). We observed the same trend in other cases also with a 2 Da shift (Figure 2B). Diagnostic ions 264 and 266 represent two lipid classes, ceramides and dihydroceramides. Using Filter results by fragments (Figure 3), we listed all the ceramide compounds present in the lipid mixture (Table 1).

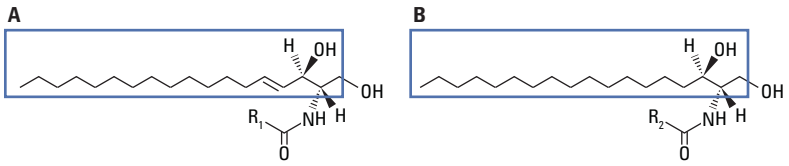

Figure 1. Ceramide (A) and dihydro-ceramide (B) structures that can lead to the formation of characteristic ions at *m/z* 264 and 266, respectively, in their MS/MS spectrum. N-acyl chains R_1 and R_2 can have same or different chain length.

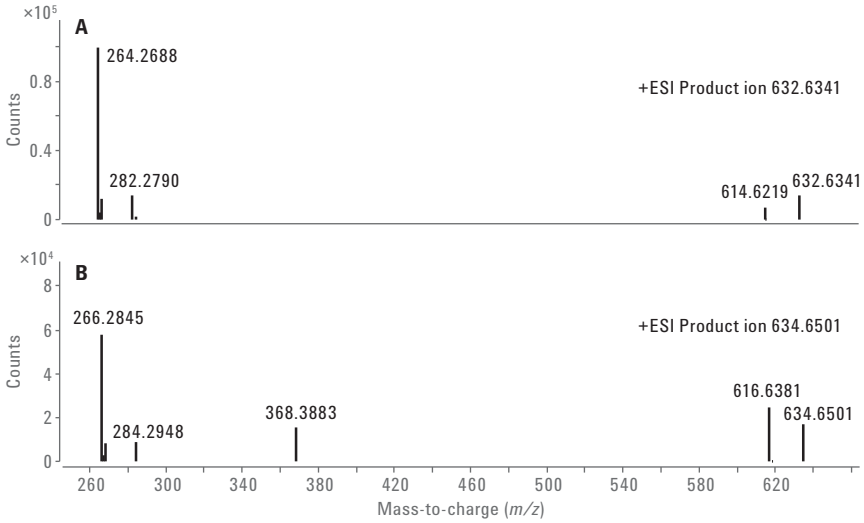

Figure 2. MS/MS spectra of ion *m/z* 520.50 showcasing a diagnostic ion at *m/z* 264.2688 (A), and *m/z* 632.6341 showcasing a diagnostic ion at *m/z* 266.2845 (B).

Figure 3 shows that retention time window and MS threshold parameters should be set after a close inspection of the chromatogram. Fragment mass information should be provided as the accurate mass. The match tolerance can influence the results significantly, and should be set according to the accuracy of the data.

PCs are another dominant class of lipids in humans. These molecules have a choline group attached to a phosphate. They exhibit a dominant fragment ion at *m/z* 184 in the MS/MS spectra in positive mode. All the glycerophosphocholines can be found by filtering the MS/MS data by *m/z* 184. The same workflow can be used by changing the fragment mass filter to 184.0732

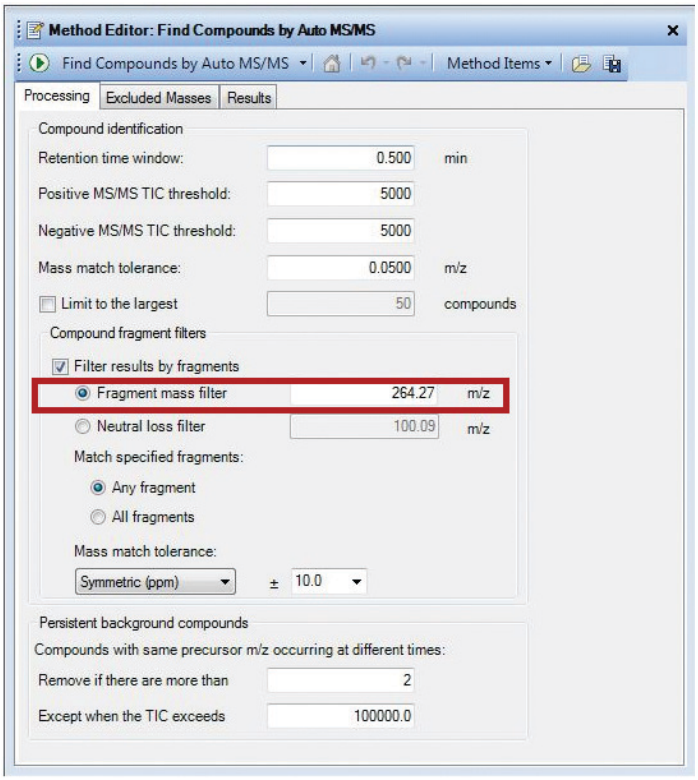

Figure 3. The Method Editor window from Agilent MassHunter Qualitative Analysis software, showing the Filter results by fragments feature.

Table 1. Partial list of results obtained after processing the data using the settings listed in Figure 3.

No.	Label	Base peak	*m/z*	RT	Height
1	Cpd 1: 30.351	264.2687	700.572	30.351	41,129
2	Cpd 2: 30.423	264.2687	682.562	30.423	33,512
3	Cpd 3: 31.527	250.2531	506.493	31.527	65,834
4	Cpd 4: 31.950	184.0739	732.557	31.95	1,957,549
5	Cpd 5: 32.716	264.2688	520.508	32.716	186,209
6	Cpd 6: 32.893	184.0737	732.557	32.893	249,240
7	Cpd 7: 32.927	264.2687	538.519	32.927	114,322
8	Cpd 8: 36.531	184.0738	786.602	36.531	166,504
9	Cpd 9: 36.817	266.2845	364.262	36.817	61,344
10	Cpd 10: 38.546	184.0738	811.669	38.546	84,878
11	Cpd 11: 38.559	387.2891	778.575	38.559	63,951
12	Cpd 12: 39.476	57.0698	611.561	39.476	65,351
13	Cpd 13: 42.319	184.0734	816.703	42.319	250,152
14	Cpd 14: 43.725	264.2688	632.634	43.725	417,833
15	Cpd 15: 43.927	264.2687	650.645	43.927	108,161
16	Cpd 16: 44.270	264.2688	614.623	44.27	77,867
17	Cpd 17: 52.291	521.4553	521.456	52.291	341,008
18	Cpd 18: 57.924	57.0698	537.524	57.924	290,505

Filtering the data by neutral loss

Prostaglandins are a group of physiologically active lipid compounds. Upon collisional activation, they give structural information through their fragments. They do not show a particular diagnostic fragment ion, but they show a characteristic neutral loss that is common in the MS/MS spectra of all the prostaglandins. Figure 4A shows the MS/MS spectra of prostaglandin E1. The protonated precursor ion is shown at *m/z* 353.2356. The labile precursor immediately loses water to form a product ion at *m/z* 335.2249. This ion, in turn, loses 100 Da to form an ion at *m/z* 235.1358. This neutral loss is a signature used to identify prostaglandins.

Similarly, PEs show a neutral loss of 141 Da in the MS/MS spectrum in positive mode. Figure 4B shows an MS/MS spectrum of a typical PE. PEs do not give structural information in positive mode, but predominantly show a neutral loss. This is sufficient to identify the polar head group of the compound in lipidomic research. In Figure 4B, the dominant peak at *m/z* 613.5544 arises due to the loss of 141 Da from the precursor ion at *m/z* 754.5737. Loss of 141 Da is a signature to identify all the PEs in a mixture.

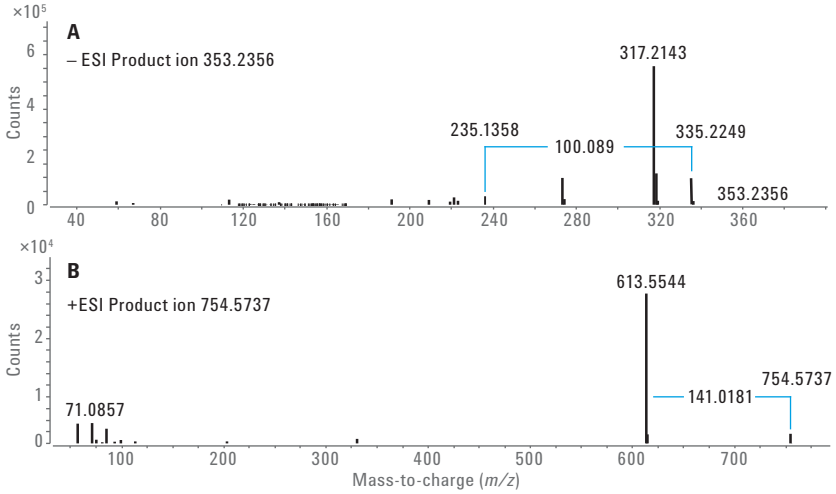

Figure 4. MS/MS spectrum of a prostaglandin (A) and PE (B) showing a neutral loss of 100 and 141 Da, respectively. The ions involved in the neutral loss are marked by the brackets.

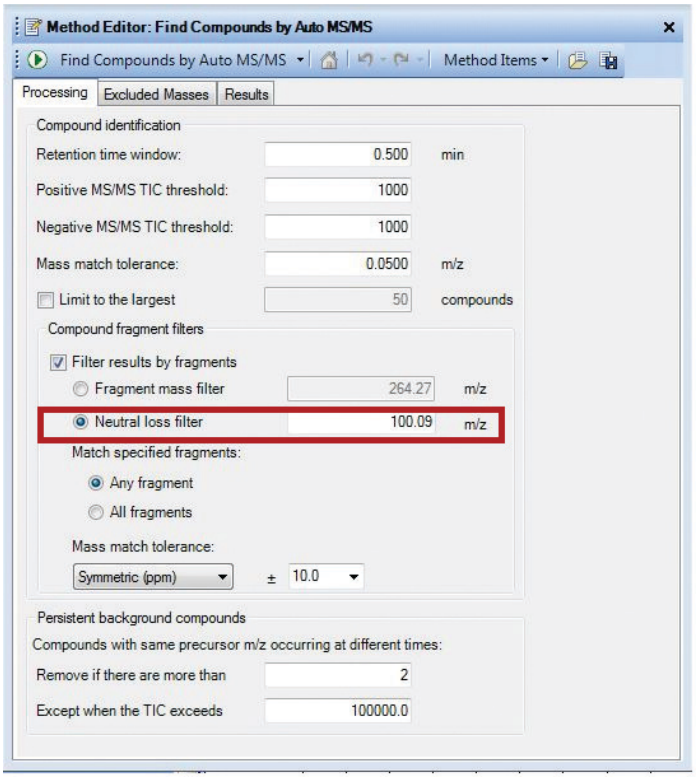

Figure 5. The Method Editor window from Agilent MassHunter Qualitative Analysis software, showing the Neutral loss filter feature.

We injected a mixture of five standard prostaglandins, and using the calculated neutral loss feature of the MassHunter Qualitative software, all could be identified. Table 2 presents a list of the compounds.

These compounds were searched against a MS/MS library, and it gave prostaglandin related hits. Figure 6 shows an example. Thus, the generated compound list can be used to identify the structure of compounds using spectral libraries such as Agilent PCDL or publicly available databases such as HMDB, LIPID MAPS, or SimLipid.

Table 2. List of the compounds generated after processing using the settings in Figure 5.

No.	Label	*m/z*	RT	Height
1	Cpd 1: 5.823	351.2195	5.823	7,057,038
2	Cpd 2: 5.872	355.2511	5.872	6,035,227
3	Cpd 3: 6.315	353.2356	6.315	3,041,384
4	Cpd 4: 6.877	351.2195	6.877	2,037,817
5	Cpd 5: 8.699	335.2249	8.699	1,822,963

The above two examples demonstrate two different types of neutral loss. In the case of prostaglandins, the neutral loss originated from a fragment ion in the MS/MS spectrum, that is, from ion *m/z* 335.2249. In the case of PEs, neutral loss occurred from the precursor ion, *m/z* 754.5737. By using the Neutral loss filter option, both features can be recognized and help in the identification of compound class.

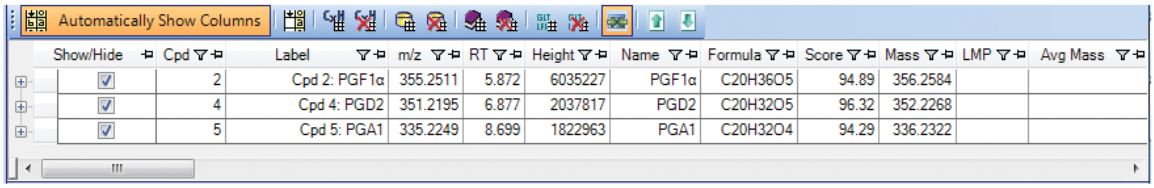

Figure 6. A screenshot demonstrating the search result for compounds 2, 4, and 5, listed in Table 2.

Conclusion

This workflow demonstrates the use of the Filter results by fragments approach to filter compounds based based on the fragment mass/neutral loss. Given the large diversity of compounds within each class of lipids, the filtering feature greatly enhances the data interpretation in lipidomics analysis.

Reference

1. Brugger, B.; *et al.* Qualitative analysis of Biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *PNAS* **1997**, *94*, 2339-2344.

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