GBSC 724
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SWATH LC-MS metabolomics

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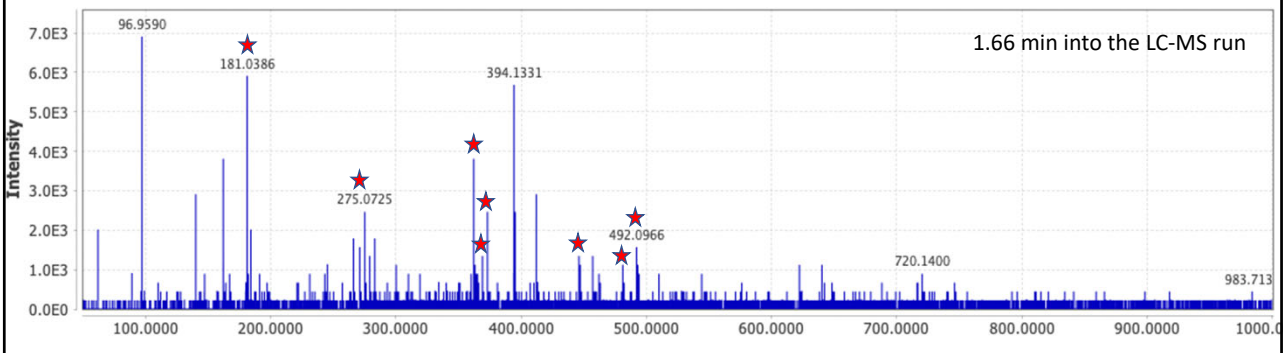
1

DDA analysis

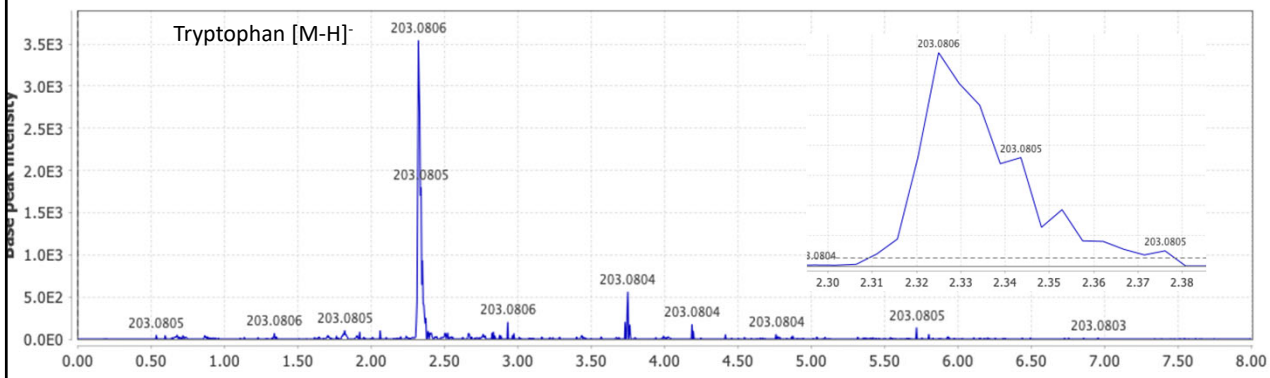
- In DDA (data-dependent analysis), the duty cycle currently lasts for 500 msec
  - In the first 100 msec, a high-resolution MS spectrum is collected
  - From this the computer decides on the top 8 molecular ions to select for sequential MSMS analyses and data collection for 50 msec per ion



2

Quantification in DDA

- This is accomplished by taking multiple MS1 spectra across a peak (4 Hz)



The peak area is ascertained by adding together the trapezoids formed from each successive data point (the beginning and the end of the peak should also be determined)

3

DDA versus DIA

- **PROs**: So, DDA collects all ions that emerge from the column during the LC run
- **CONs**: The MSMS (or MS2) data is sampled and therefore is by no means complete. Also, we cannot quantify using the MS2 data, since the sampling is somewhat arbitrary.
- **DIA (data-independent analysis)** overcomes this issue by collecting MS2 (and MS1) data almost continuously
  - **SWATH-MS (Sequential Window Acquisition of all Theoretical Mass Spectra)** is a specific variant of DIA

4

Building a SWATH method

- In DDA, the molecular ion is selected with a 1 Da window (for singly charged ions)
- In SWATH, the mass window width is opened up considerably and a series of MS2 spectra at increasing mass values are collected **without regard to whether ions are present** in that mass window range
  - This is fixed SWATH
  - Let's say that the observable ions are from *m/z* 50-850, then we could sequentially collect 32 MSMS spectra starting at *m/z 50* in 25 *m/z* SWATHS.
  - If each MSMS spectrum requires 50 msec acquisition time, how long does this take to complete one cycle?
  - Does this coordinate with LC conditions where a peak is 3 sec wide?

5

Doing SWATH-MS analysis requires compromise

- **If the peak widths are 3 sec wide, then we must collect 10 data points per SWATH duty cycle, i.e., every 300 msec, 100 msec of which is devoted to collection of the MS1 spectra.**
  - So, now with 200 msec available, the 32 SWATH windows can be for only 6 msec – lowered sensitivity compared to the 50 msec MS2 collections in DDA
- **How to go forward?**
  - Less (and hence wider) mass windows (can we resolve the MS2 ions coming from each molecular ion?)
  - Slow the chromatography down (to generate wider peaks, thereby buying time)
    - But this consumes time – and lowers the peak intensities

6

Variable SWATH

- **The distribution of molecular ions that are present in each sample type is not uniform**
- **Therefore, for a particular sample type, it is worth running an initial analysis to determine that distribution**
- **The SWATHs then can be weighted to reflect the distribution and do optimal data collection**
- **See more on SWATH-MS at https://sciex.com/content/dam/SCIEX/pdf/tech-notes/all/SWATH-for-Metabolomics-Library-Comparison.pdf**

7

Class exercise – due Friday, Feb 4

- You've been asked to set up/design a SWATH MS experiment
- The current LC method creates 2.5 sec wide peaks
- The new mass spectrometer is 20 times more sensitive than the previous one
- It also is capable of collecting data at 100 Hz, i.e., every 10 msec
- Preliminary data suggest that the ions of interest fall in the range of m/z 50-850
- Can you design a SWATH analysis using these limitations and assure your mentor that the SWATH assay will be more sensitive than the DDA assay they are currently using? Show the math!!

8