UAB Workshop on the analysis of LC-MS data
December 17th, 2013

Identifying and validating metabolites

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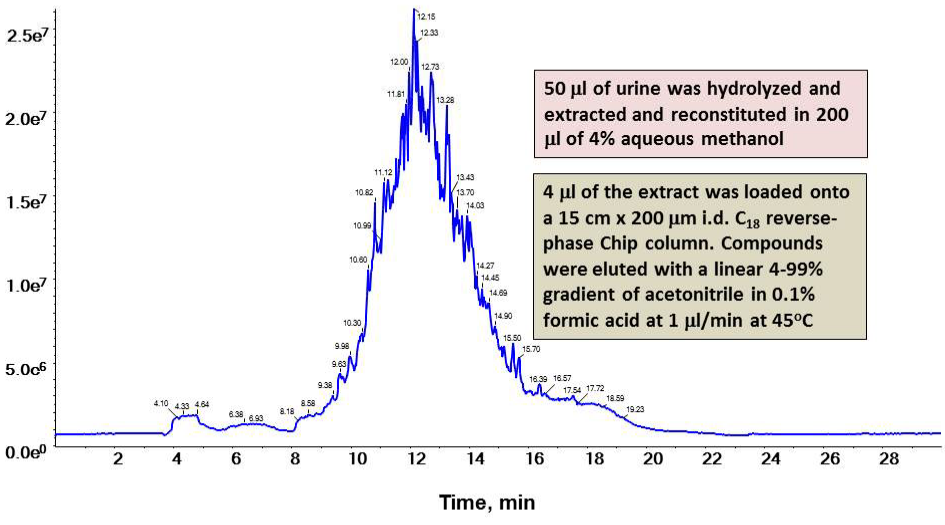
CCTS

Total ion current of extracted urine

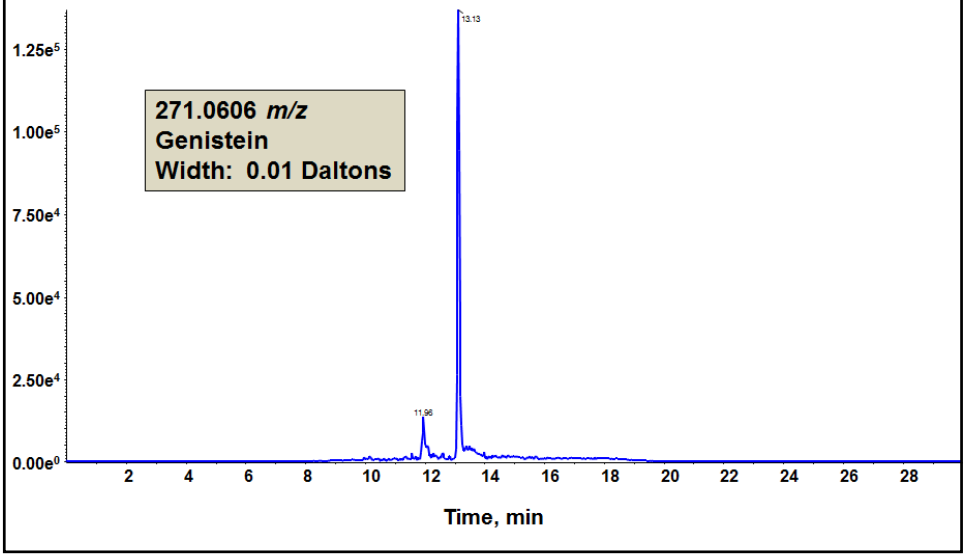


50 µl of urine was hydrolyzed and extracted and reconstituted in 200 µl of 4% aqueous methanol

4 µl of the extract was loaded onto a 15 cm x 200 µm i.d. C_{18} reverse-phase Chip column. Compounds were eluted with a linear 4-99% gradient of acetonitrile in 0.1% formic acid at 1 µl/min at 45°C

The collected data represent a data library that can be searched and experiments conducted AFTER data collection

Shrinking the mass window allows MS detection of Genistein directly in urine



Have I just measured genistein?

$[M+H]^+$ = 271.060 *m/z*

Corresponds to $C_{15}H_{10}O_5$

How?

Calculating exact mass

- **C = 12.000000 15 x 12.000000 = 180.000000**
- **H = 1.007825 10 x 1.00782503 = 10.078250**
- **O = 15.994914 5 x 15.9949146 = 79.974573**
- **Monoisotopic molecular weight= 270.052823**
- **Monoisotopic ion $[M+H]^+$ = 271.060099**

Identifying an ion

- **METLIN** – a database of metabolites maintained by investigators at Scripps Research Institute
  - http://metlin.scripps.edu
- Supplemented by the **Human Metabolite Database** (HMDB) maintained by David Wishart at the University of Alberta
  - http://www.hmdb.ca

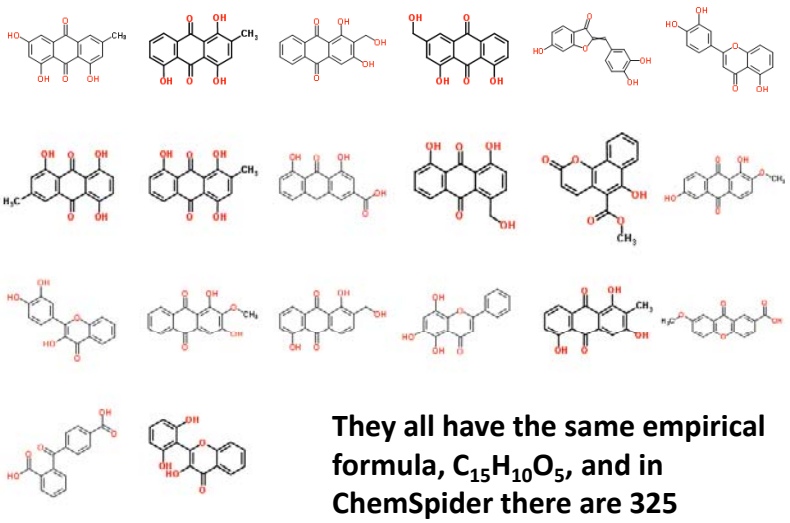
A more detailed database

- **Royal Institute of Chemistry has an extensive database of compounds**
  - http://www.chemspider.com/
  - Select “Advanced Search”
  - Select by “Search by Properties”
  - Uncheck “Molecular Formula”, “Molecular Weight”, “Nominal Mass”, “Average Mass”
  - Under “Monoisotopic mass” enter the *m/z* value of the ion, leave the error at 0.001; in the next pull down select “M+H” – finally, select “Search”

ChemSpider output

- **Several items are in the report**
  - Record ID
  - Compound structure
  - Empirical formula
  - Exact mass of the compound (not the ion)
  - Reference sources

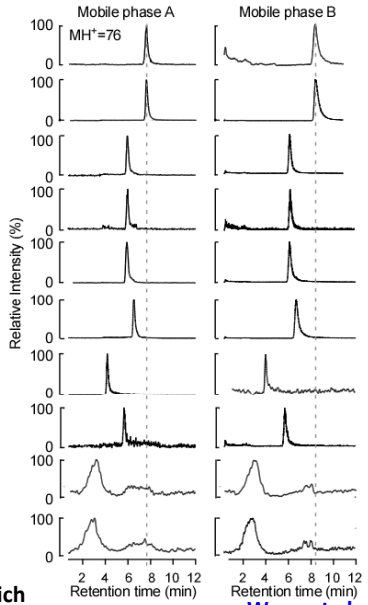
Compounds in ChemSpider



They all have the same empirical formula, $C_{15}H_{10}O_5$, and in ChemSpider there are 325

Identifying the *m/z* 76 ion by retention time

Candidate plasma analyte m/z=76		MH^+
Trimethylamino N-oxide (TMANO)	$(CH_3)_3NOH$	76
1-Amino-2-propanol	$CH_3CH(OH)CH_2NH_3$	76
2-Amino-1-propanol	$CH_3CH(NH_3)CH_2OH$	76
3-Amino-1-propanol	$NH_3CH_2CH_2CH_2OH$	76
Methylamino-ethanol	$CH_3NH_2CH_2CH_2OH$	76
Glycolamide	$HOCH_2CONH_3$	76
Hydroxy-guanidine	$HONHC(NH_3)=NH$	76
Glycine	NH_3CH_2COOH	76
N-Isopropyl-hydroxylamine	$(CH_3)_2CHNH_2OH$	76



Candidate compounds from Sigma-Aldrich

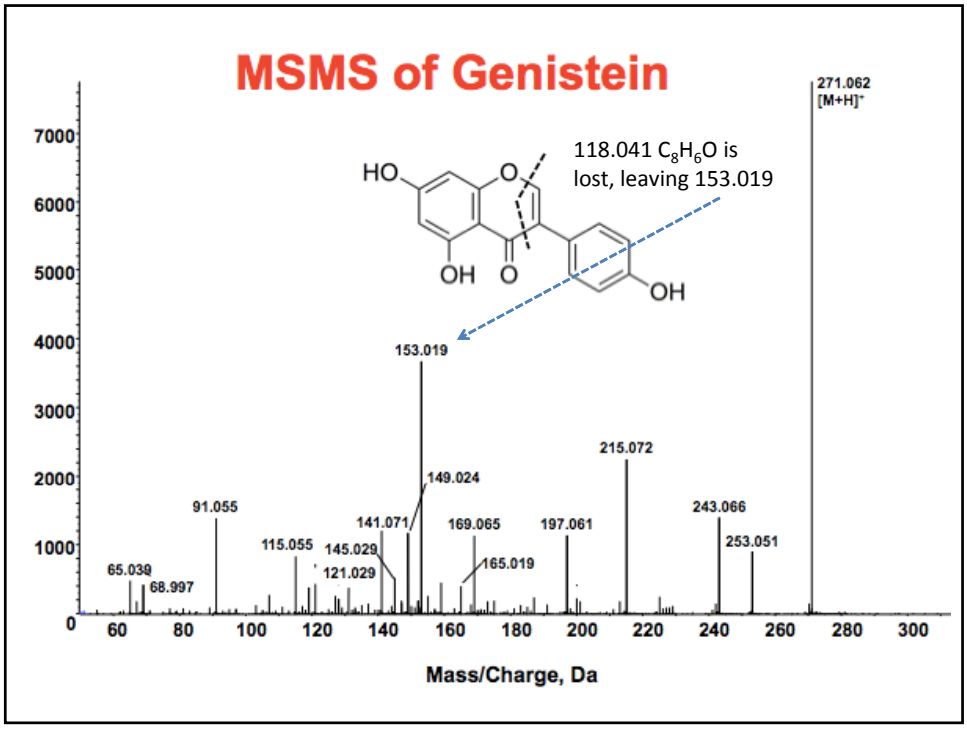
Wang et al., Nature (2011)

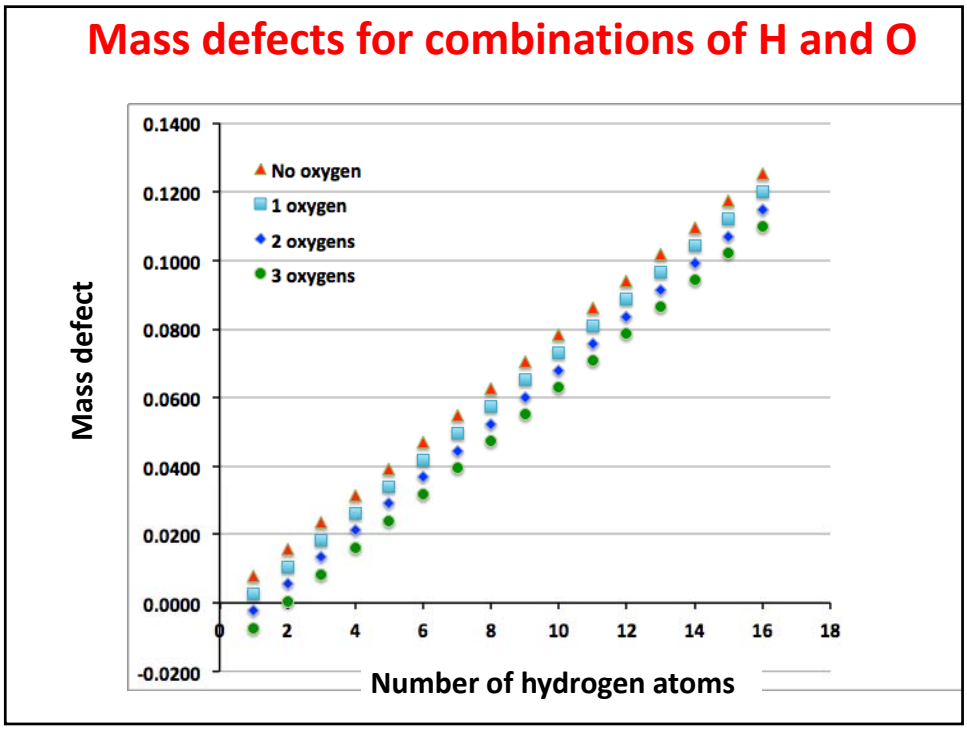
Important points to make

- **The ion was noted as *m/z* 76**
  - **Must contain 1 N (or another odd number) atom**
  - **3 of the 9 possibilities would have had a different *m/z* value**
    - **Glycine and glycolamide ($C_2H_6NO_2$) – *m/z* 76.039**
    - **Hydroxyguanidine (CH_6N_3O) – *m/z* 76.051**
    - **Others ($C_3H_{10}NO$) – *m/z* 76.076**
- **The chromatographic property of the metabolite is as important as the mass spec data**

Sorting out the possibilities

- **Many of the compounds are synthetic**
  - So, delete those that are not biological
- **Carry out MS/MS**
  - Select the molecular (precursor) ion
  - Accelerate this ion into gas causing heat-induced dissociation to form product ions
  - Separate ions in a second mass spectrometer analyzer


MSMS of Genistein
118.041 C_8H_6O is lost, leaving 153.019
HO
O
OH
O
OH
271.062 [M+H]+
153.019
215.072
149.024
91.055
243.066
141.071
169.065
197.061
253.051
115.055
145.029
121.029
165.019
65.039
68.997
7000
6000
5000
4000
3000
2000
1000
0
60
80
100
120
140
160
180
200
220
240
260
280
300
Mass/Charge, Da


Mass defects for combinations of H and O
No oxygen
1 oxygen
2 oxygens
3 oxygens
0.1400
0.1200
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
-0.0200
0
2
4
6
8
10
12
14
16
18
Mass defect
Number of hydrogen atoms

Selecting the isobaric metabolite

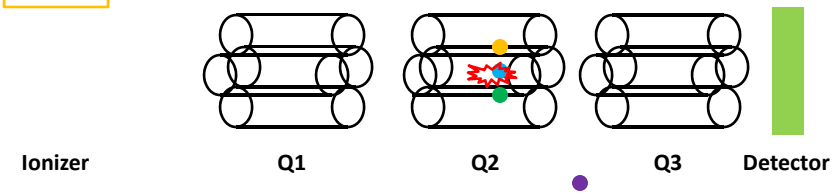
- Obtain a sample of the possible compounds
- Perform MSMS experiment
- Compare each of their MSMS spectra to the unknown metabolite
- Run the sample containing the metabolite and the isobaric possibilities with two different LC analytical methods to confirm identical elution times
- Then run a MRM experiment

Multiple reaction ion monitoring

- Based on the mass transition from the precursor ion and a unique product ion
- Combined with a LC method
- Can be carried out with a triple quadrupole mass spectrometer
- Better on a Q-TOF since ALL the product ions can be measured with high mass accuracy

Multiple reaction ion monitoring

Quantitative analysis of a metabolite in a complex mixture carried out using a triple quadrupole instrument



Based on precursor ion/product ion pair(s)

Courtesy, John Cutts

Pseudo-multiple reaction ion monitoring

Quantitative analysis of a metabolite in a complex mixture carried out using a Q-TOF instrument