

PROFILING BOURBONS AND AMERICAN WHISKEYS USING UHPLC/QTOF-MS

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Defining Whiskey

- Whiskey is a spirit produced by distilling grain based mashes; grains in common use include:
 - corn
 - rye
 - wheat
 - barley (malted or un-malted)
- Whiskeys are distilled using pot stills or in column or continuous stills
- Most whiskeys are aged in wooden casks for some period of time, which is often legally defined

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- Blended whiskeys, craft distilled whiskeys

Whiskey profiling

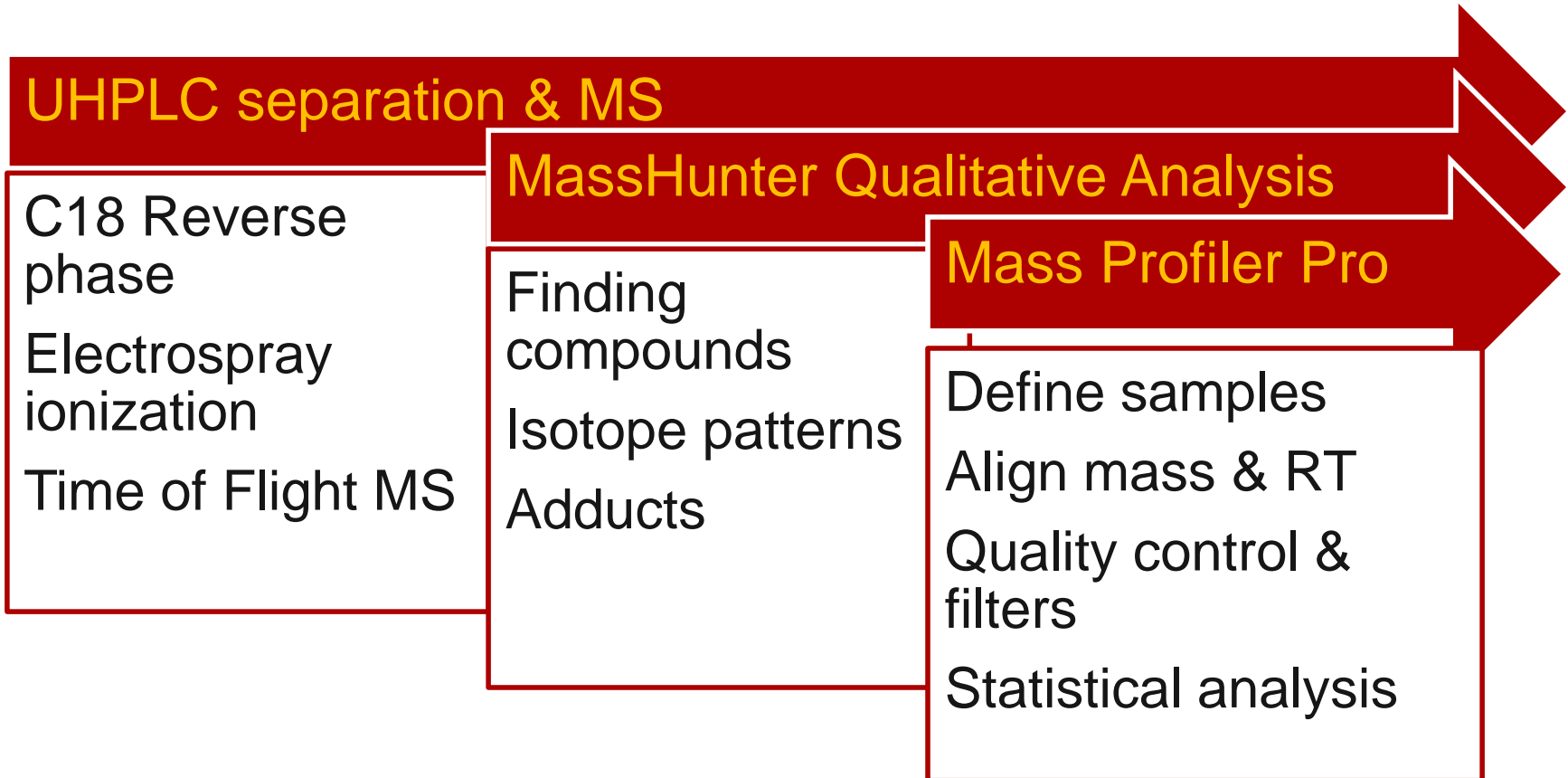
- Confirmation of authenticity
 - Isotopic ratios for source of ethanol
 - Volatile profiles by GC/MS
 - Spectroscopic methods—near IR and mid-IR
 - UHPLC/QTOF
- Quality assurance/process improvement
 - Evaluation of raw materials
 - Impact of fermentation/distilling process
 - Evaluation of cooperage
 - Changes in whisky composition during aging

UHPLC/QTOF

- High performance liquid chromatography coupled to high resolution mass spectrometer
- The time of flight (TOF) mass spectrometer provides accurate mass data
- The quadrupole (Q) provides MS/MS capability



Profiling workflow



Whiskey samples

- 67 whiskeys covering a broad range of American whiskeys
 - 41 Bourbon whiskeys
 - 13 Rye whiskeys
 - 6 Tennessee whiskeys
 - 7 Other American whiskeys
- Commercial product purchased at retail stores and reputable online merchants

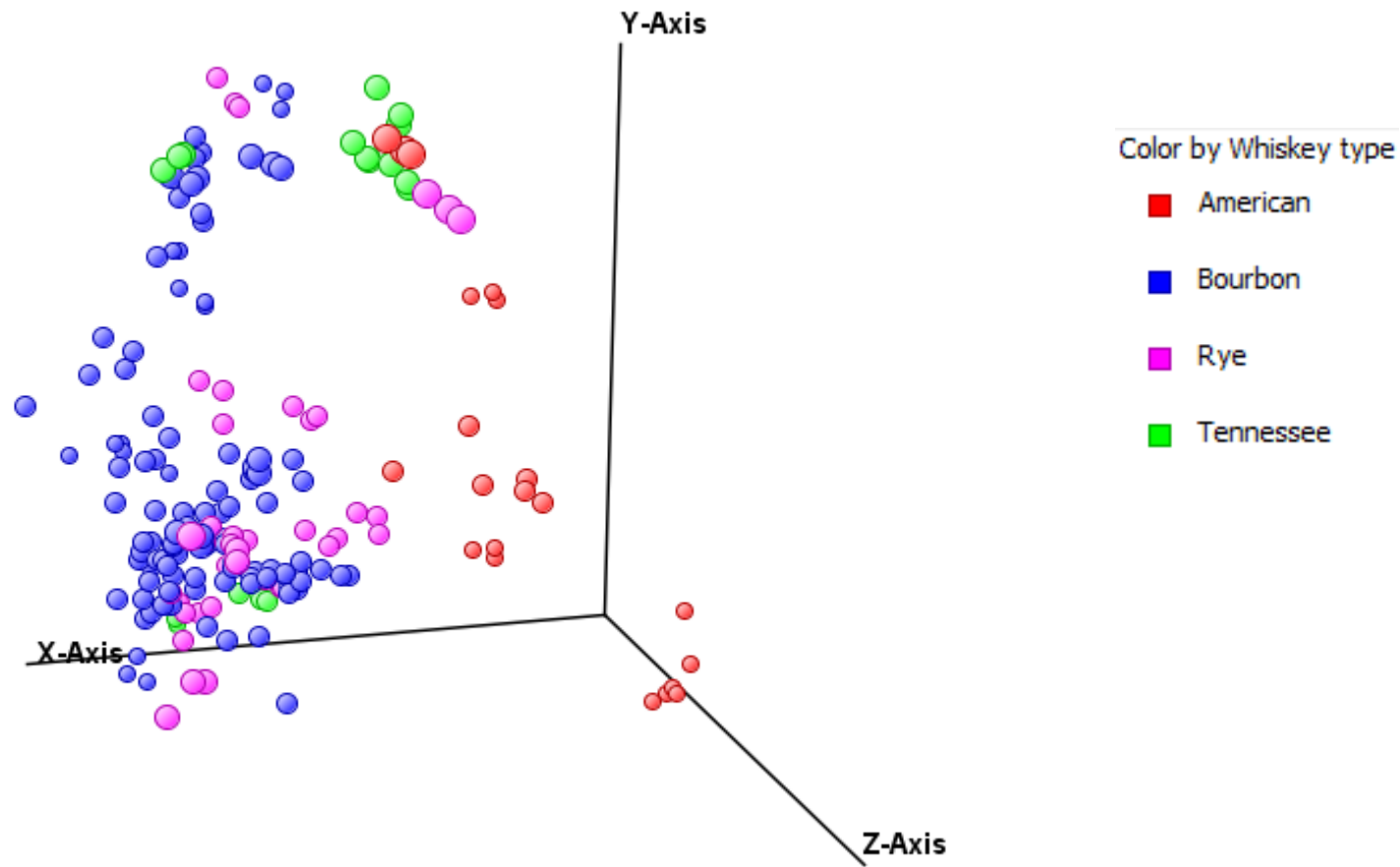
Whisky profiling

- Samples were run neat, in triplicate
- ESI, negative mode, 100-1100 m/z
- Sample order was randomized and all samples were run over a two day period
- Each sample was also analyzed in an untargeted MS/MS mode (20V collision energy)

Whiskey profiling

- The initial set of ~3000 entities (accurate mass & RT) across the samples was narrowed down by screening for:
 - Presence across replicates
 - Minimum abundance
 - ANOVA for significance
- A set of 40 entities was selected to differentiate across the whiskeys
 - An accurate mass database search of these masses found compounds in these chemical classes:
 - Wood related volatile phenols and polyphenols
 - Terpenes and related compounds
 - C6 and higher alcohols, esters and acids

3D Principal Component Analysis (PCA) of 67 American whiskeys

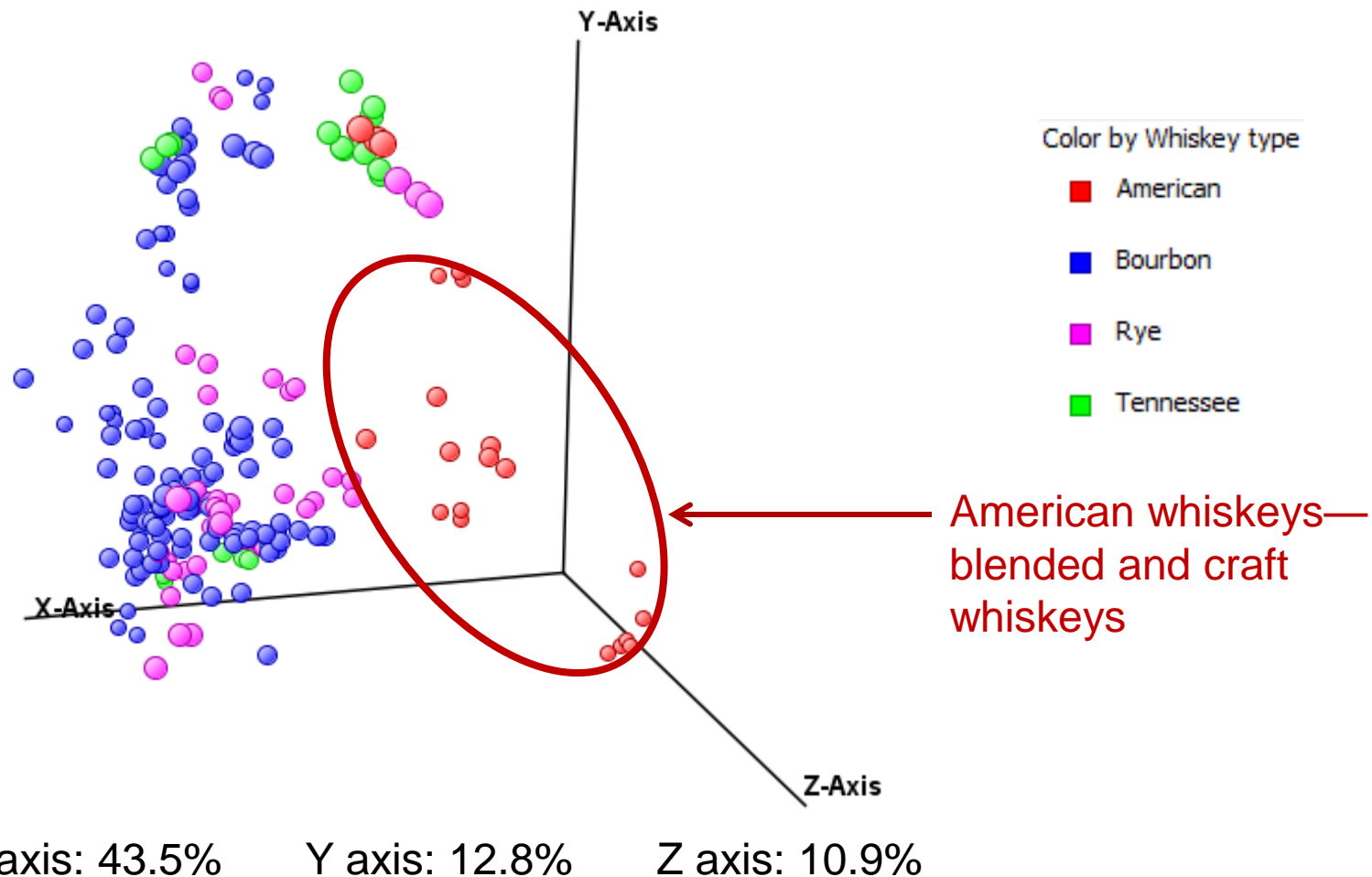


X axis: 43.5%

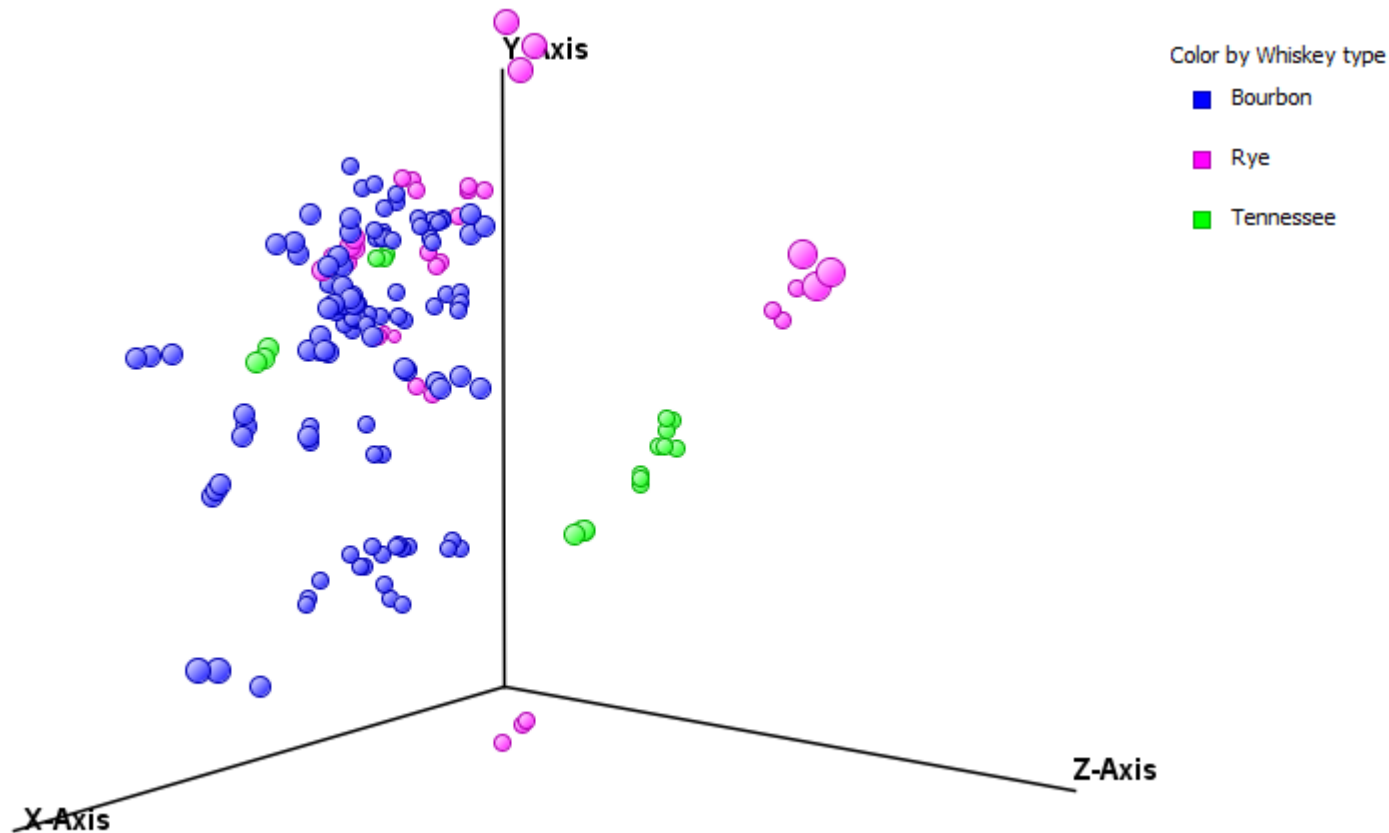
Y axis: 12.8%

Z axis: 10.9%

3D Principal Component Analysis (PCA) of 67 American whiskeys



3D PCA of 60 Bourbon, Rye and Tennessee whiskeys

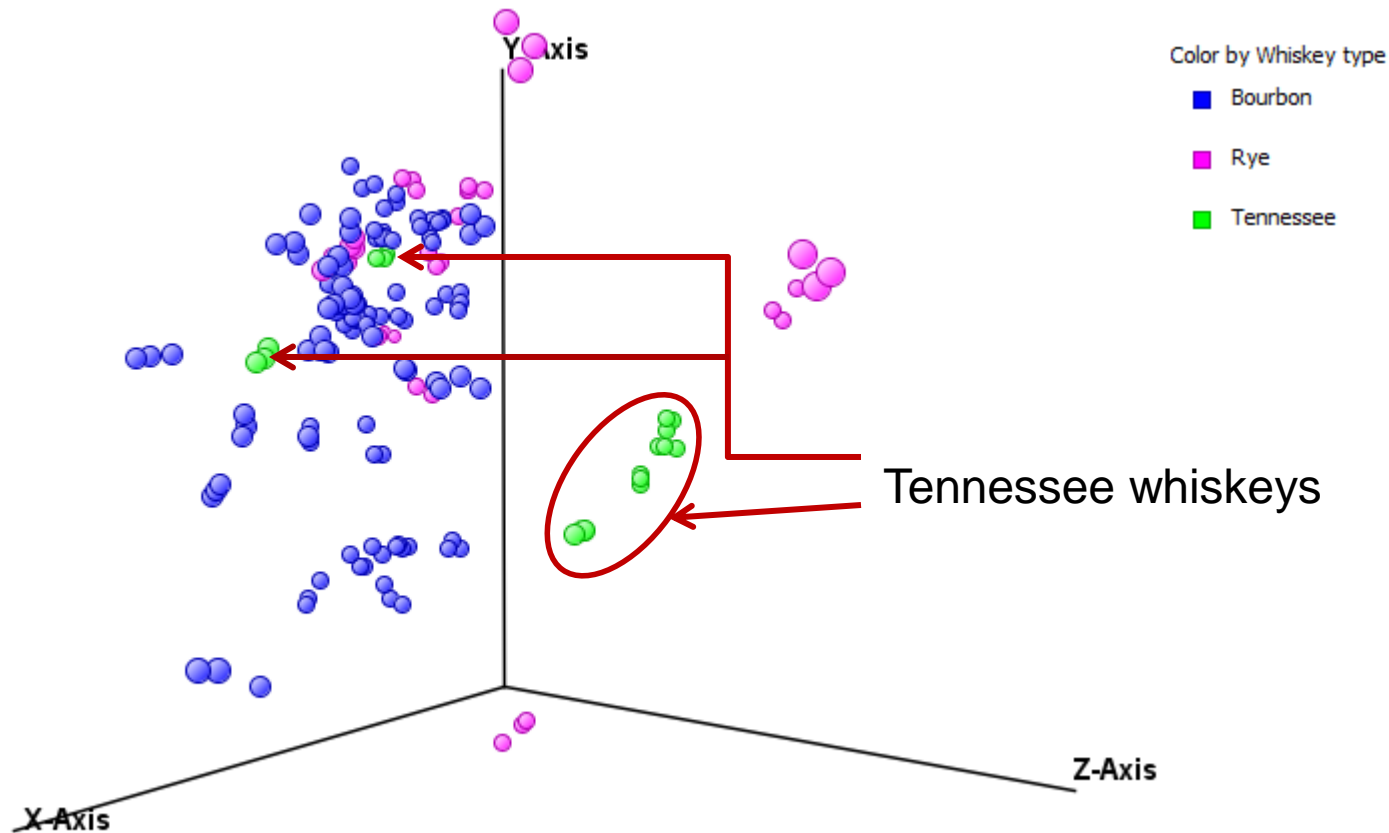


X axis: 31.9%

Y axis: 15.4%

Z axis: 9.1%

3D PCA of 60 Bourbon, Rye and Tennessee whiskeys

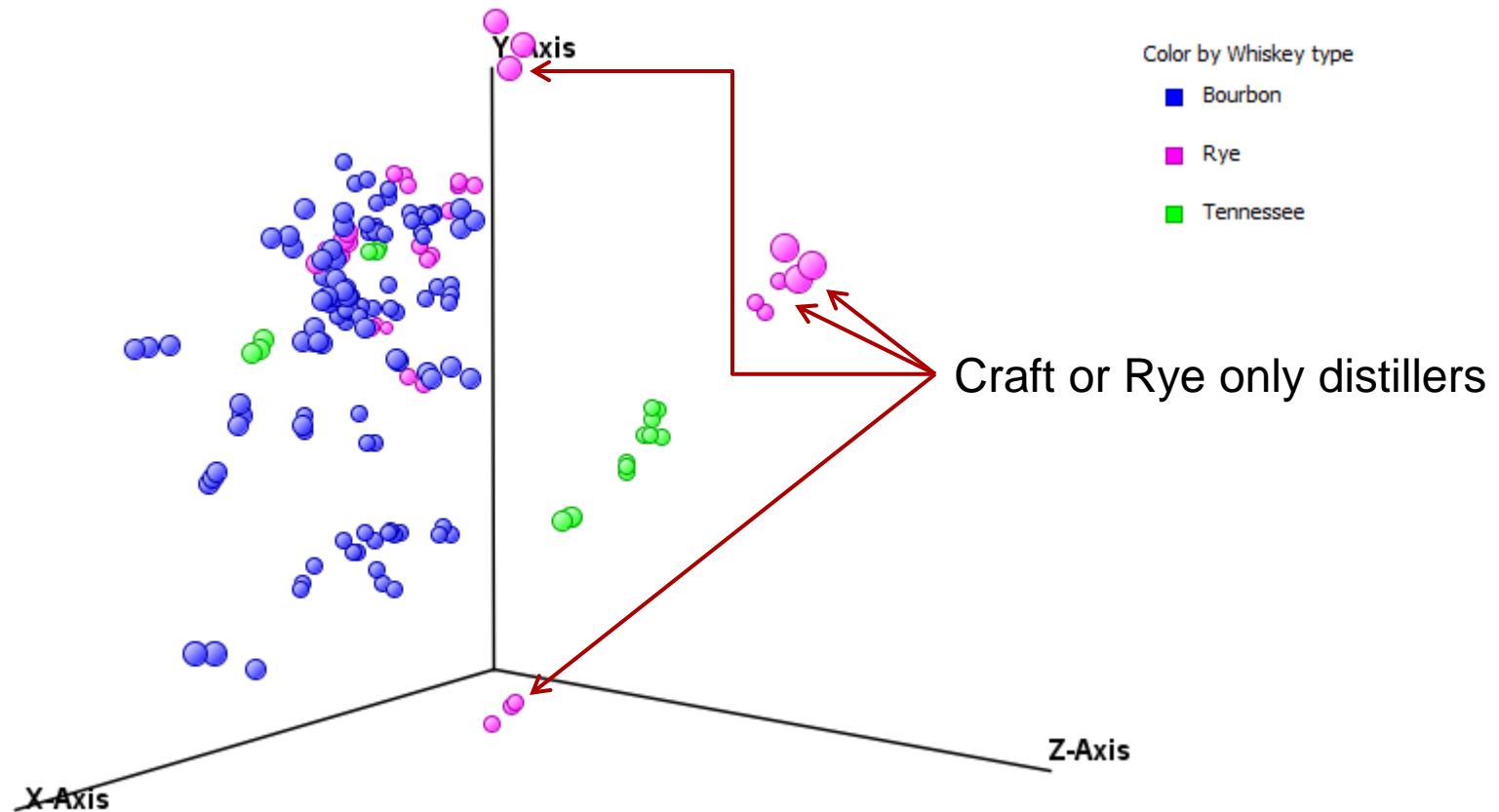


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Profiling

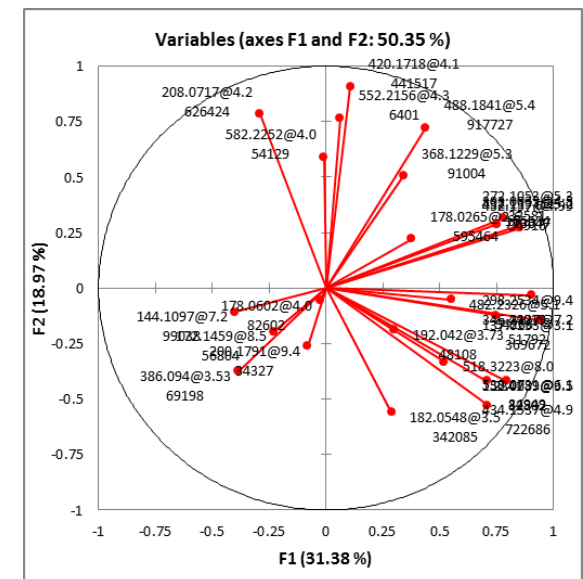
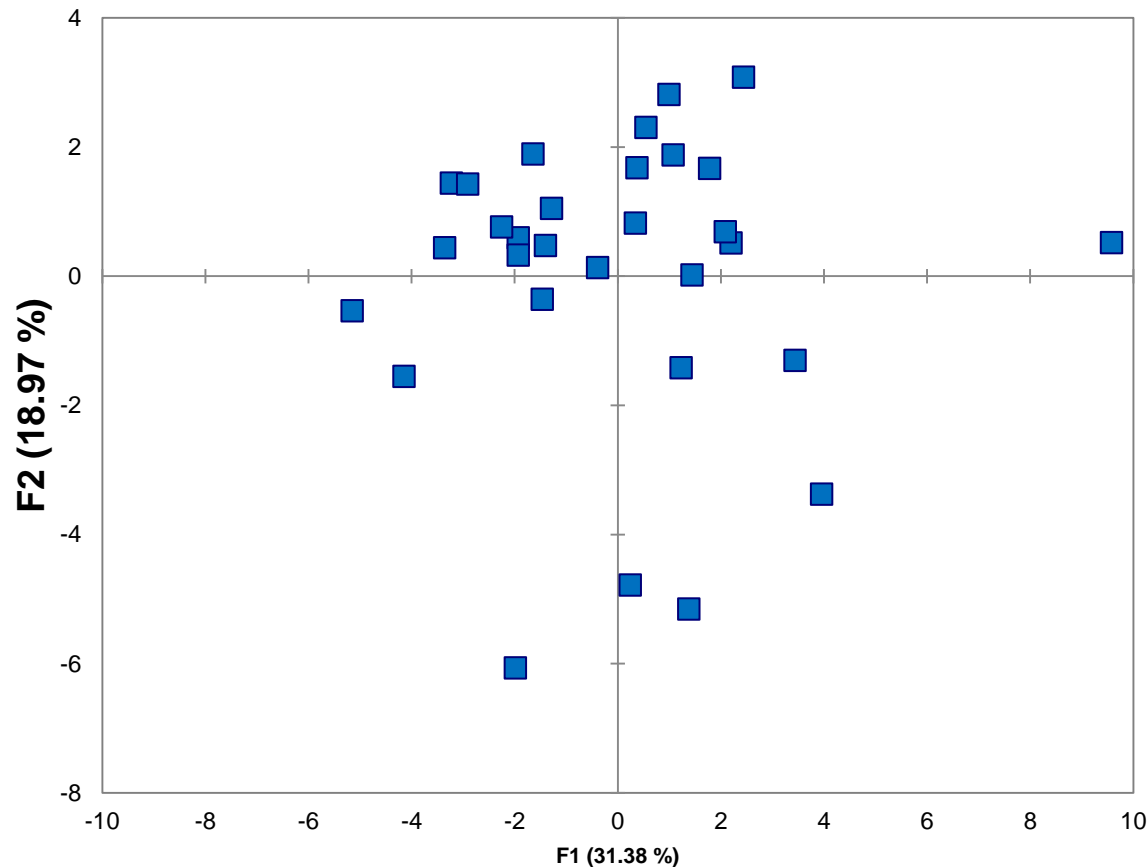
- Profiling can be targeted or untargeted
 - If specific compounds of interest are known, these can be used for targeted profiling—e.g., use of oak aroma related volatiles for profiling barrels
 - In many cases, profiling is a discovery process—it is used to find compounds to screen samples within a set
- It is not always necessary to identify the compounds which drive a profile
 - While the accurate mass data can be sufficient to provide a chemical formula, in many instances there are multiple compounds with the same formula and accurate mass

Identifying the entities used to differentiate these whiskeys

- Search Scripp's Metlin Metabolomics and Tandem MS database
 - Accurate mass MS database
 - Has MS/MS data for an ever-increasing number of compounds
- Comparison with other published MS and MS/MS results in whiskeys, other spirits and oak barrels
 - Much of the published work in on volatile composition, but there is some data on semi volatile and non-volatile composition as well.

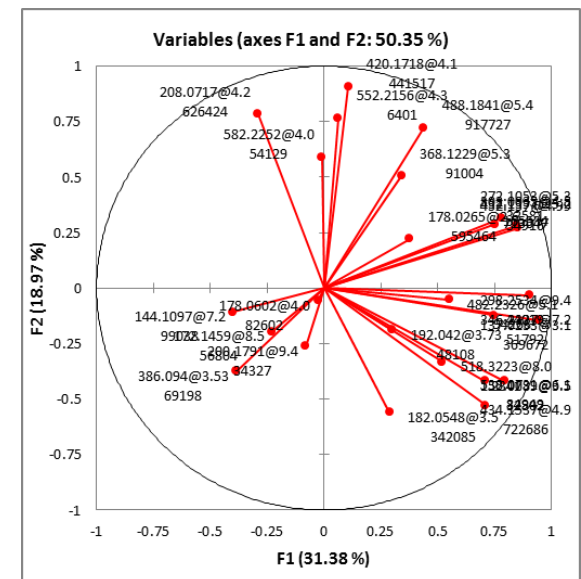
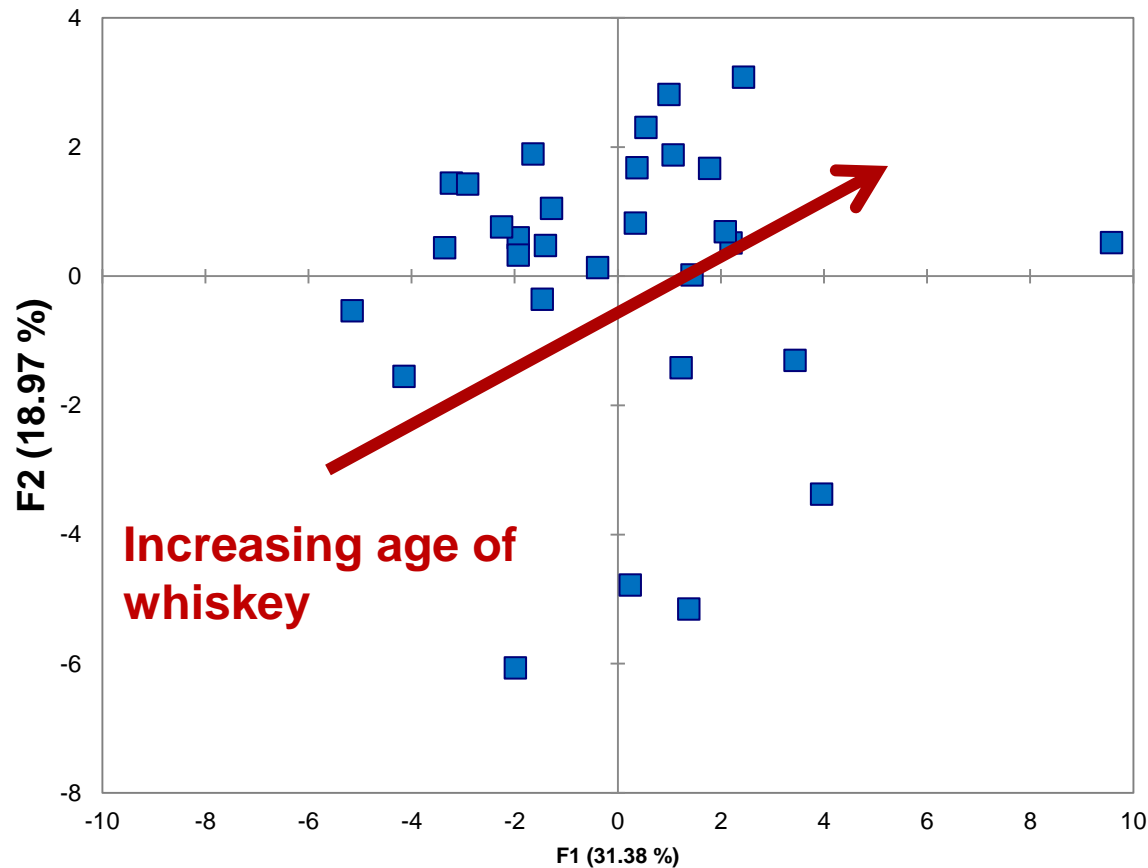
2D PCA of Bourbons only, averaged data by individual bourbons (37 entities)

Observations (axes F1 and F2: 50.35 %)



2D PCA of Bourbons only, averaged data by individual bourbons

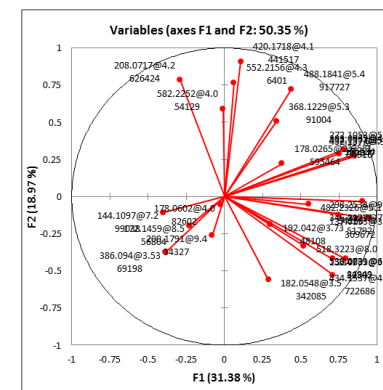
Observations (axes F1 and F2: 50.35 %)



Compounds associated with longer aging of the whiskey

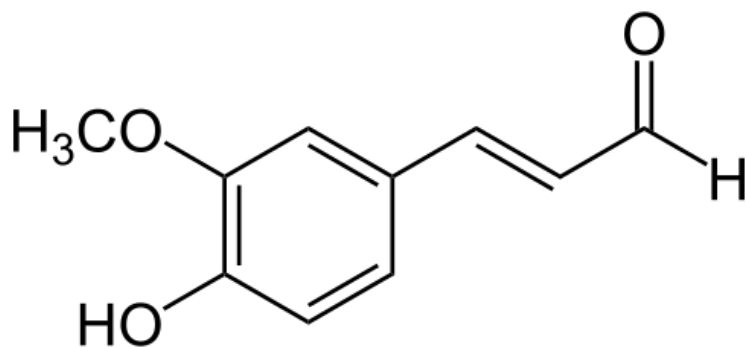
Compound	Mass	RT	Source
Esculetin	178.0265	2.66	Metlin MS/MS
Ellagic acid	302.0035	4.52	Metlin + standard
Octadecanoic acid, dihydroxy- or hydro-peroxy	346.2327	7.25	Metlin MS/MS
Unknown flavonoid	368.1229	5.39	Mass Hunter
Lyoniresinol	420.1718	4.14	MacNamara ¹
Methylated Lyoniresinol	434.1526	4.96	MacNamara ¹
Lyoniresinol xyloside	552.2156	4.36	MacNamara ¹

¹McNamara, et al, LC/GC Europe, 2011

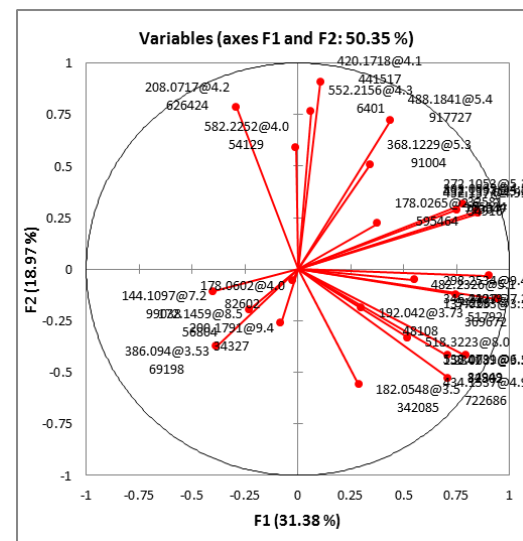


Compounds associated with lesser aged whiskeys

Compound	Mass	RT	Source
Octanoic acid	144.1097	7.30	Metlin MS/MS
Decanoic acid	172.1459	8.56	Metlin MS/MS
Coniferaldehyde	178.0502	4.08	Metlin MS/MS
Syringaldehyde	182.0548	3.53	Metlin MS/MS
Dodecanoic acid	200.1791	5.39	Metlin MS/MS

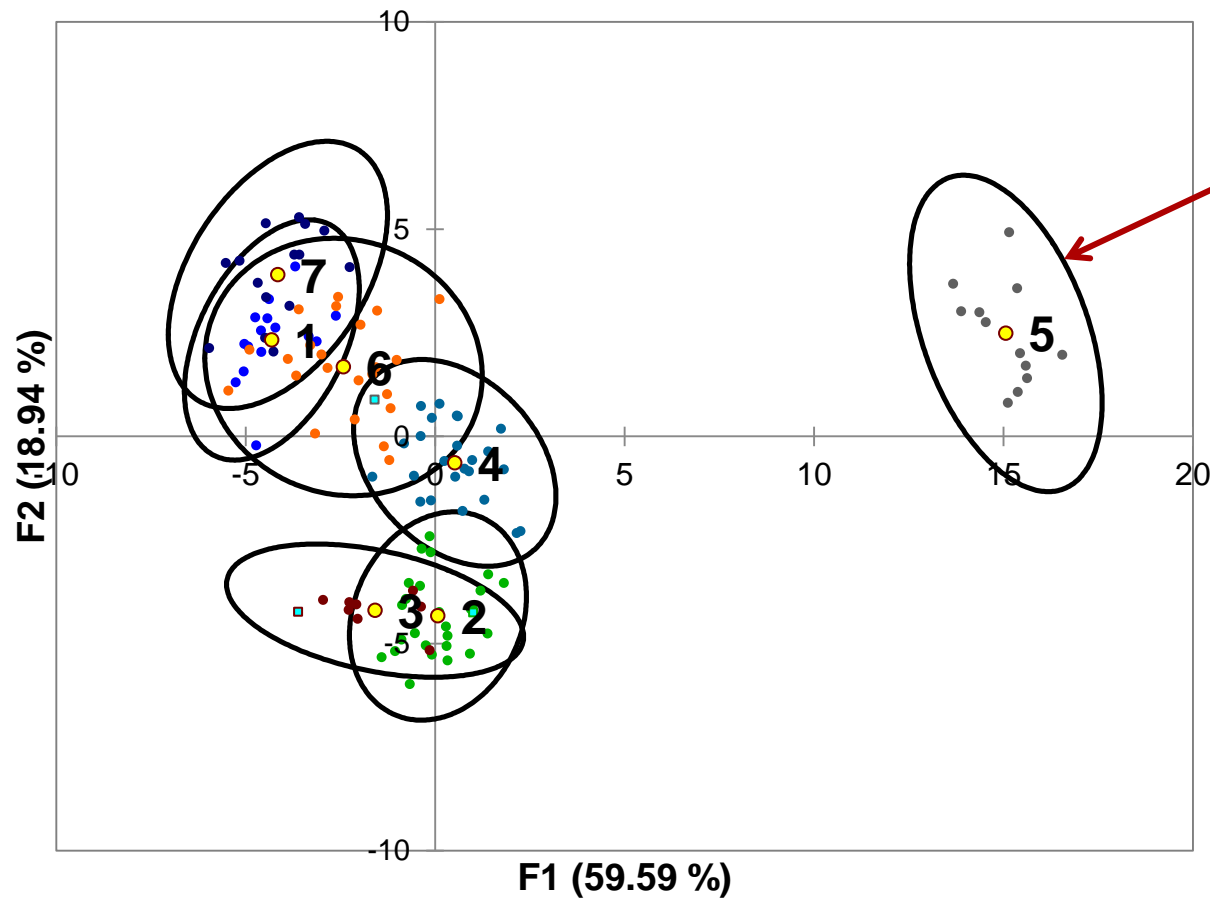


Coniferaldehyde



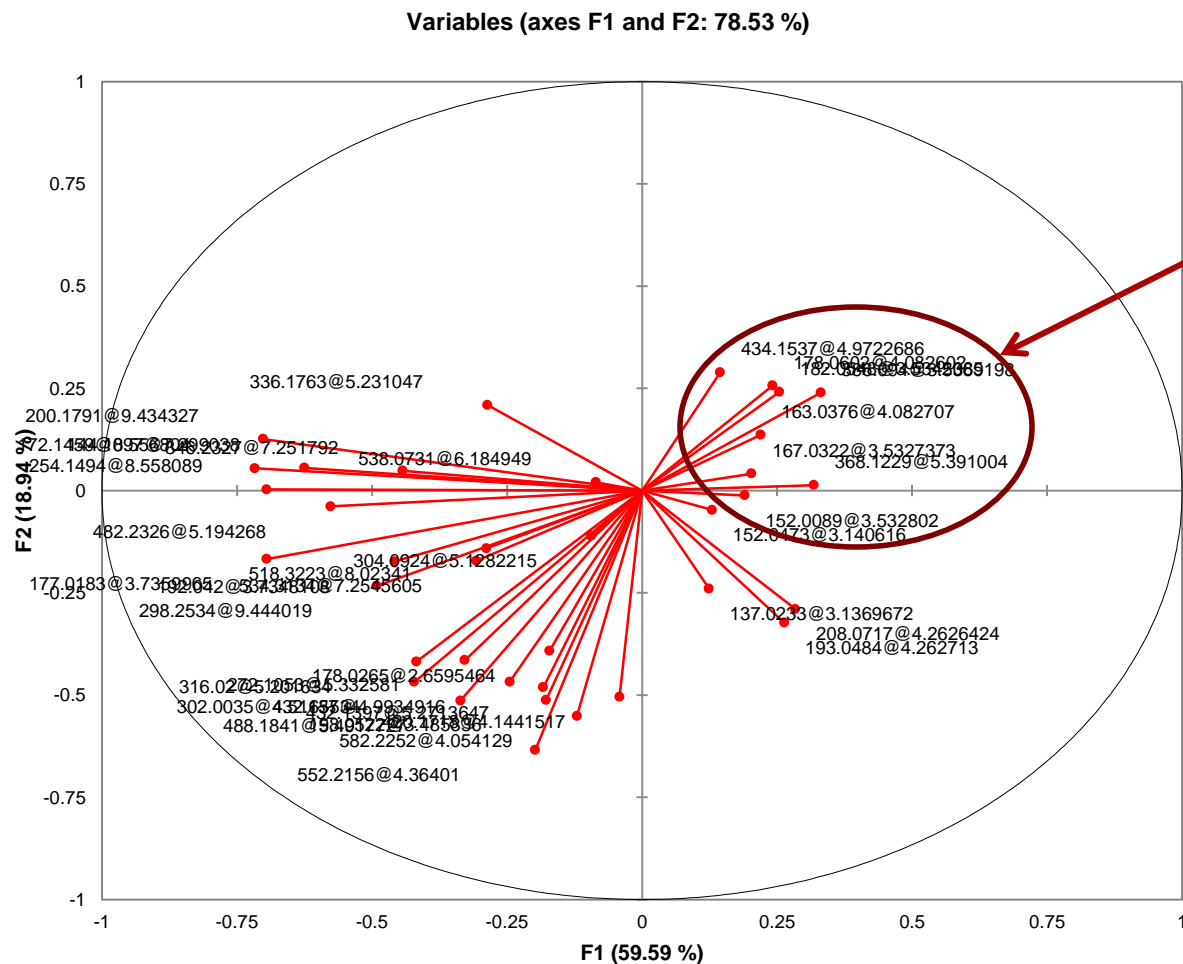
Discriminant Analysis of Bourbons by Producer

Observations (axes F1 and F2: 78.53 %)



95% confidence ellipses

Associated compounds by producer



Compounds associated with producer #5's whiskeys

Compound	Mass	RT	Source
Unknown	163.0376	4.08	Mass Hunter
Unknown	167.0322	3.53	Mass Hunter
Coniferaldehyde	178.0502	4.08	Metlin MS/MS
Syringaldehyde	182.0548	3.53	Metlin MS/MS
Flavonoid	368.1229	5.39	Metlin MS/MS
Flavonoid	386.0940	3.53	Metlin MS/MS
Methylated Lyoniresinol	434.1537	4.97	MacNamara ¹

¹McNamara, et al, LC/GC Europe, 2011

Conclusions

- UHPLC/QTOF-MS was used to profile 67 whiskeys from a broad range of origins and ages
 - Bourbon, Rye and Tennessee whiskeys were well differentiated from blended American whiskeys
 - Some Tennessee and some Rye whiskeys could be differentiated from Bourbons
 - Identification of compounds which drive these differentiations is ongoing, using MS/MS results to compare with database and previously published MS/MS data

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