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Chemical Profiling and Differential Analysis of Whiskies using Exactive™ GC Orbitrap™ GC-MS

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Overview

- Introduction to whisky adulteration
- Technology solution
- Study details
- Results
- Conclusions

Introduction

£5 Billion

$120 Billion

- Chemical profiling of whisky is required:
  - Quality control of whisky for consistent product
  - Understand how aging and storage impacts the final taste and odour of the finished product.
  - Combat adulteration/counterfeiting is a significant threat.

*Scotch Whisky Association, 2015.
**Distilled Spirits Council USA, 2015.

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Whisky Adulteration

- Three common types of adulteration:

1. Artificial colouring to simulate the aging process. Can also be achieved by heating during the aging process to speed up the colouration.

2. Substitution of an alternative spirit with chemicals artificially added to simulate whisky flavours and colour. No control over these chemicals.

3. Refilling labelled bottles with either a cheaper genuine whisky or an artificial whisky. i.e., 5 year whisky into a 18 year whisky labelled bottle, or blended whisky into single malt labelled bottle.

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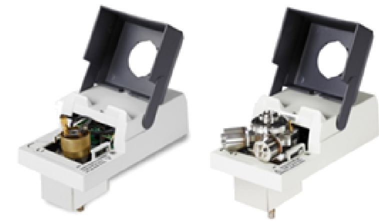
Orbitrap™ GC-MS: The Technology

Orbitra™p mass analyzer

Incredible HRAM performance

Extended Dynamic Range



Thermo Scientific™ Trace 1310 GC system

Unique modular injector and detector design

Short cycle time

Thermo Scientific™ ExtractaBrite™ ion source technology

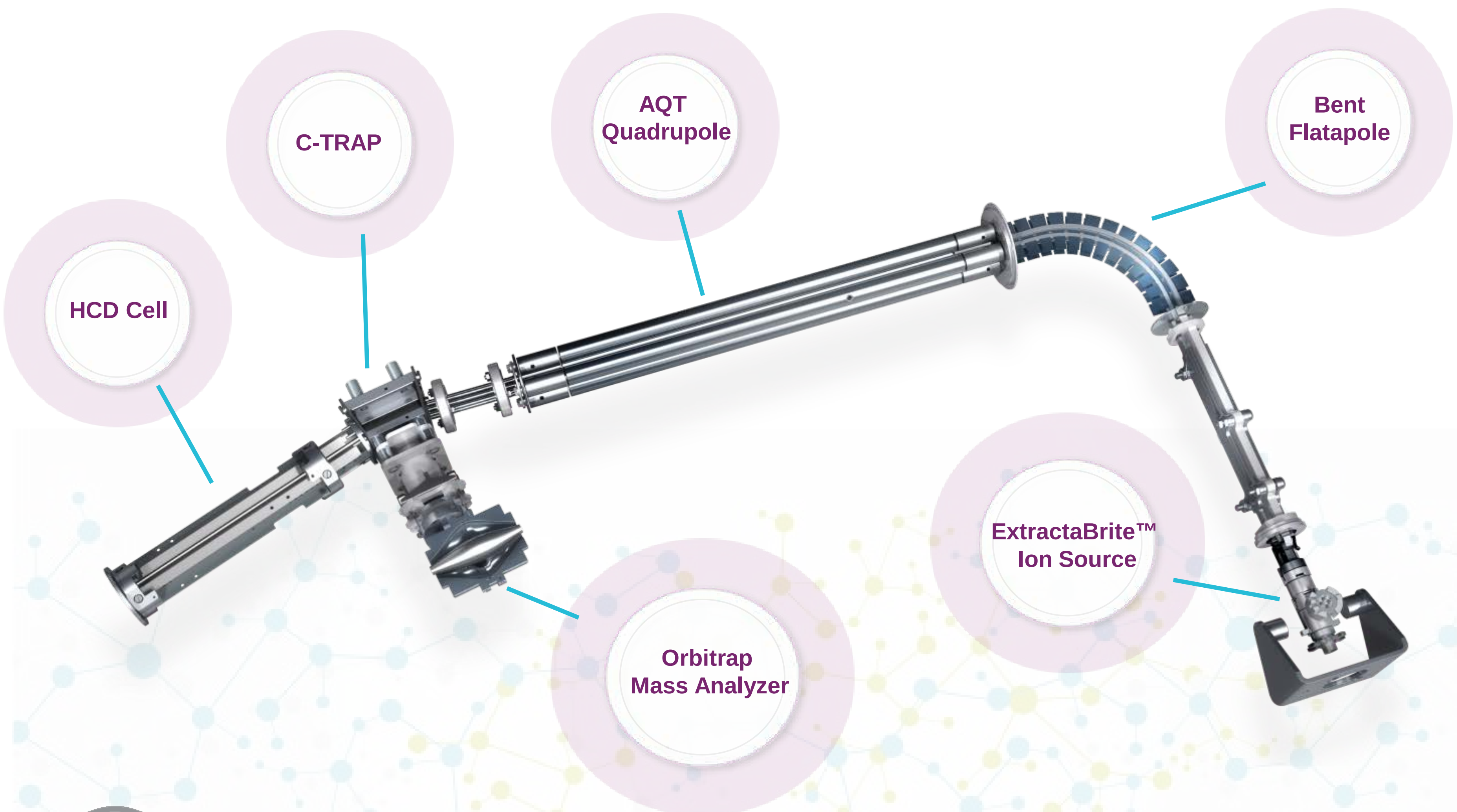
Routine grade robustness

Patented RF lens

Removable without breaking vacuum through VPI

Vacuum-free column replacement through VPI

Bringing GC and Orbitrap™ Technology Together



Two Options in the Orbitrap™ GC-MS Family

Redefining Routine GC-MS

RP 60,000 (FWHM @ *m/z* 200)

EI/CI; Full-scan; Timed-SIM

Thermo Scientific™ Exactive™ GC system

Thermo Scientific™ Q Exactive™ GC system

Unprecedented Depth in Analysis

RP 120,000 (FWHM @ *m/z* 200)

EI/CI; Full-scan, Timed-SIM

MS/MS capability

Analytical Approach in This Proof of Concept Study

- Are there any chemical differences between whisky samples?
  - Bourbon or Scotch Whisky
  - USA or Scotland, Highland or Lowland
  - 10,15, 18 year aging

- Approach was to run full scan analysis under generic GC conditions and use software tools to identify.
  - if there are differences: Using statistical software i.e, Compound Discoverer™
  - what the differences are:
    Using software tools including NIST libraries, deconvolution software and elemental composition and fragment matching software

Experimental – Whisky Profiling

Experimental - Samples

- 9 whisky extracts in ethyl acetate analysed 4 times in random order
- Pool sample prepared from 20 µl each whisky
- Run at 60,000 resolution on Thermo Scientific™ Q Exactive™ GC system

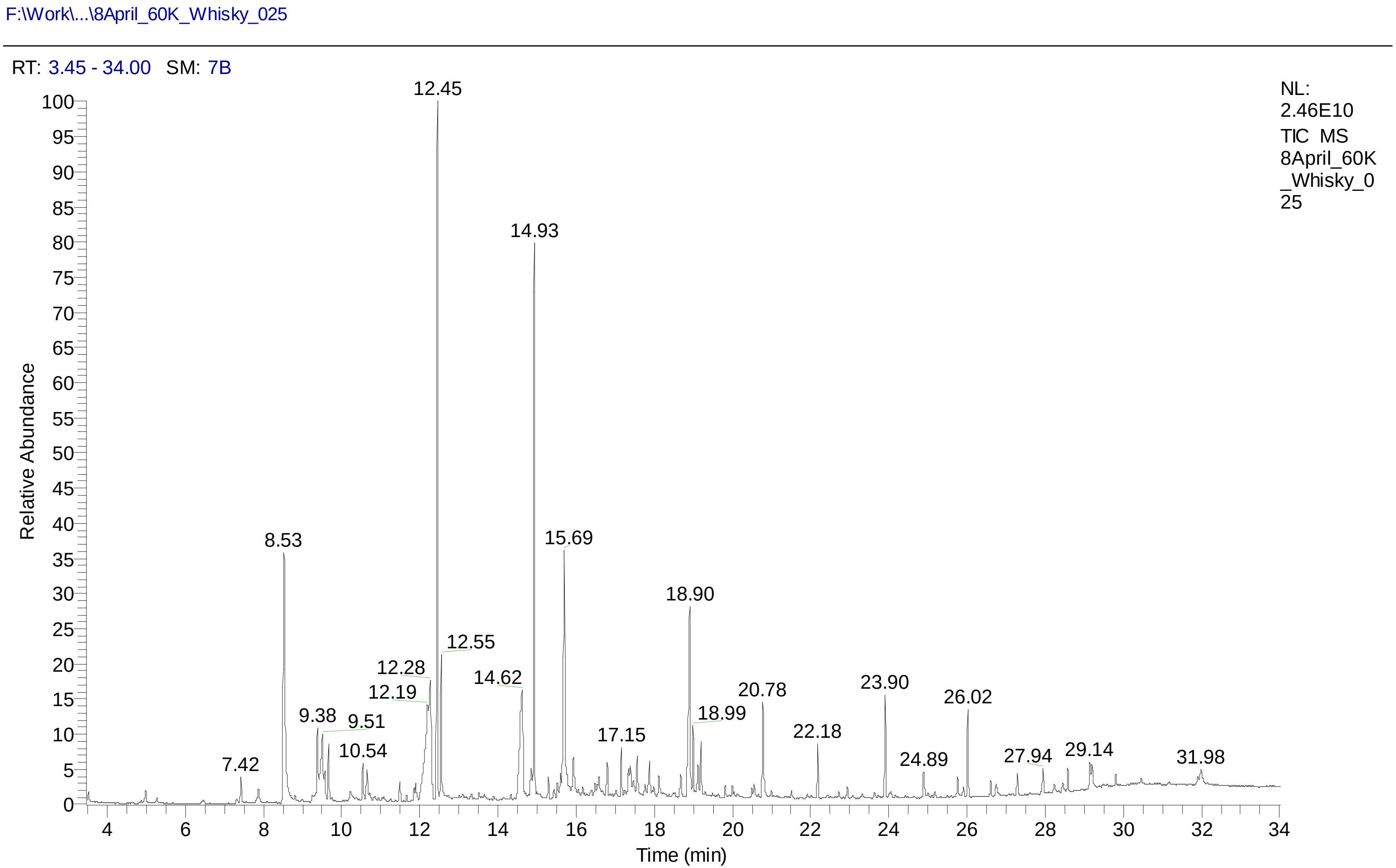
Sample ID	Type	Age	Country of Origin	Region
2263	Single	12	Scotland	Lowlands
2264	Single	18	Scotland	Lowlands
2265*	Single	NAS	Scotland	Lowlands
2281	Single	10	Scotland	Campbeltown
2282	Single	15	Scotland	Campbeltown
2283	Single	15	Scotland	Campbeltown
2284	Single	12	Scotland	Highland
2285	Single	18	Scotland	Highland
2295	Bourbon	-	USA	Kentucky

Experimental – Instruments

- Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
- Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ Trace™ 1310 GC.
- Data was processed using the Compound Discoverer 2.1 Peaks investigated in Thermo Scientific™ TraceFinder™ deconvolution software.

Results – Whisky Profiling

TIC Whisky Sample

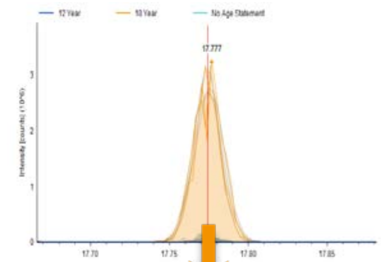


Differential Analysis Workflow

import .raw data
(full scan EI or CI)

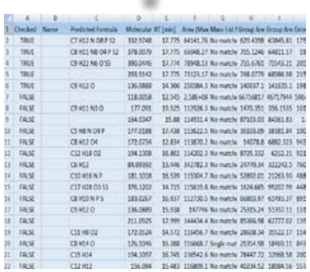
Experiment definition
(sample grouping)

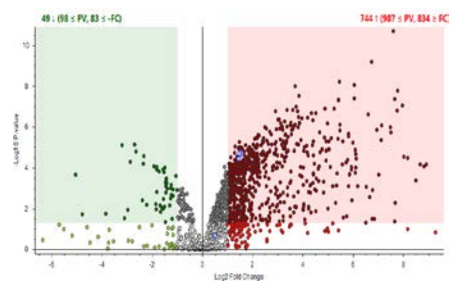
Data processing
(peak alignment & extraction)

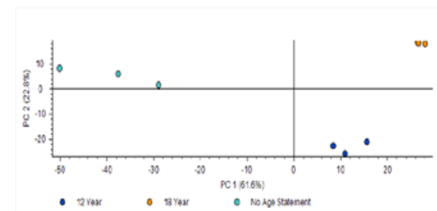


ID in TraceFinder

Statistical analysis





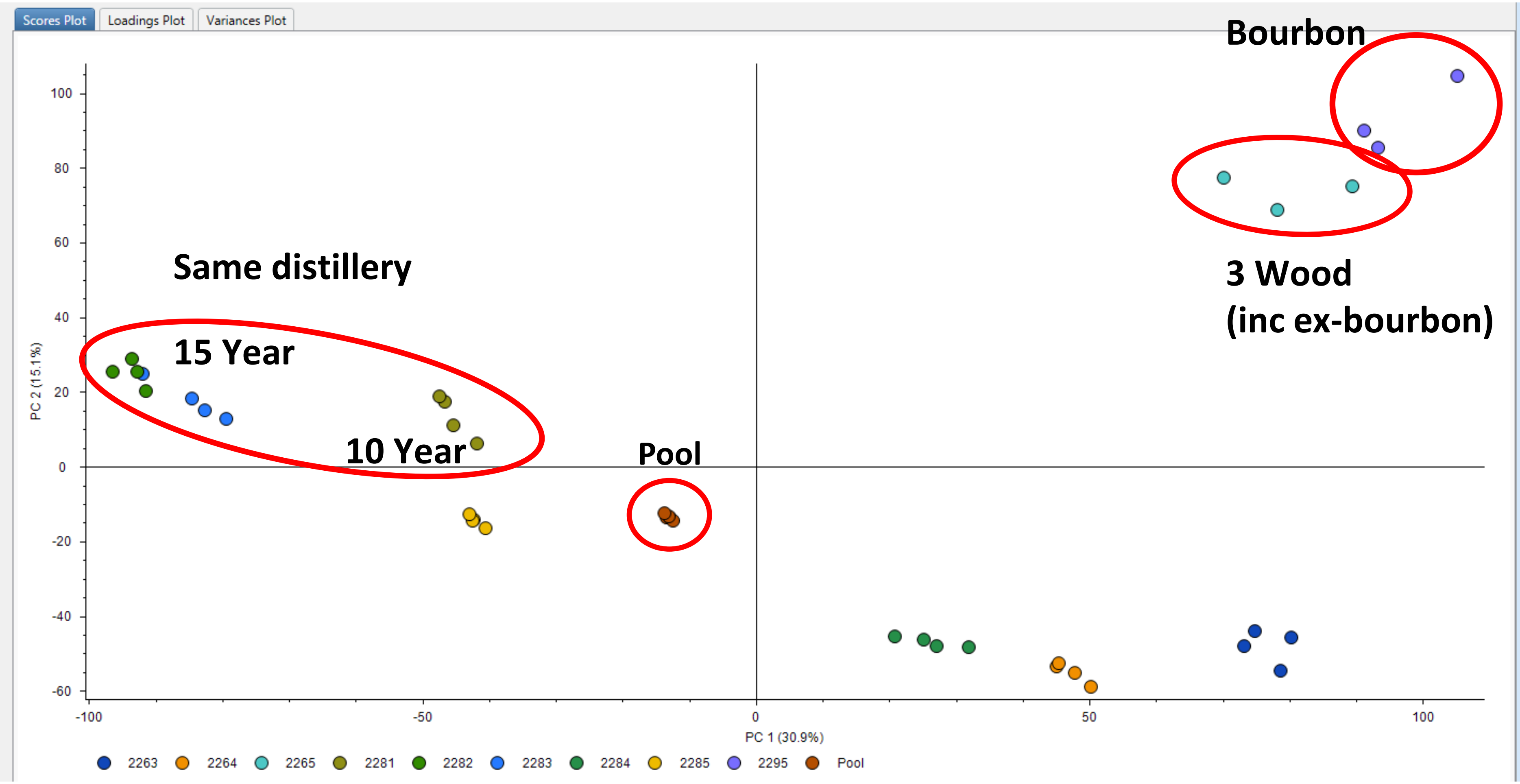


Data export

Data review
(based on %CV, p-values)

PCA from Compound Discoverer

Technical replicates in good agreement and clear differences between samples

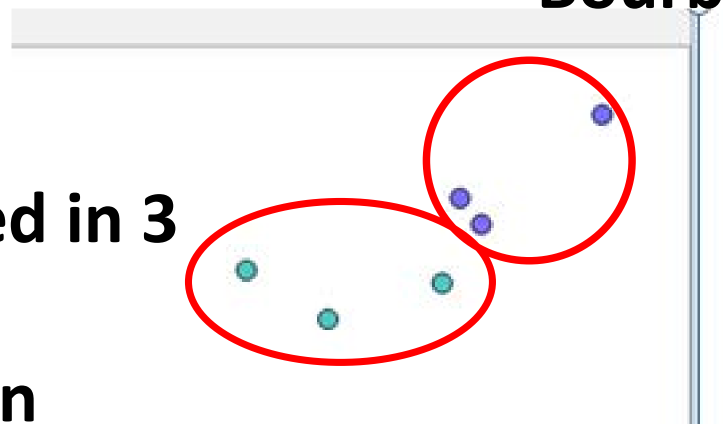


Taking a Closer Look at the Data

Initial approach is to investigate the differences in the bourbon and 3 wood aged from other whiskies

Bourbon

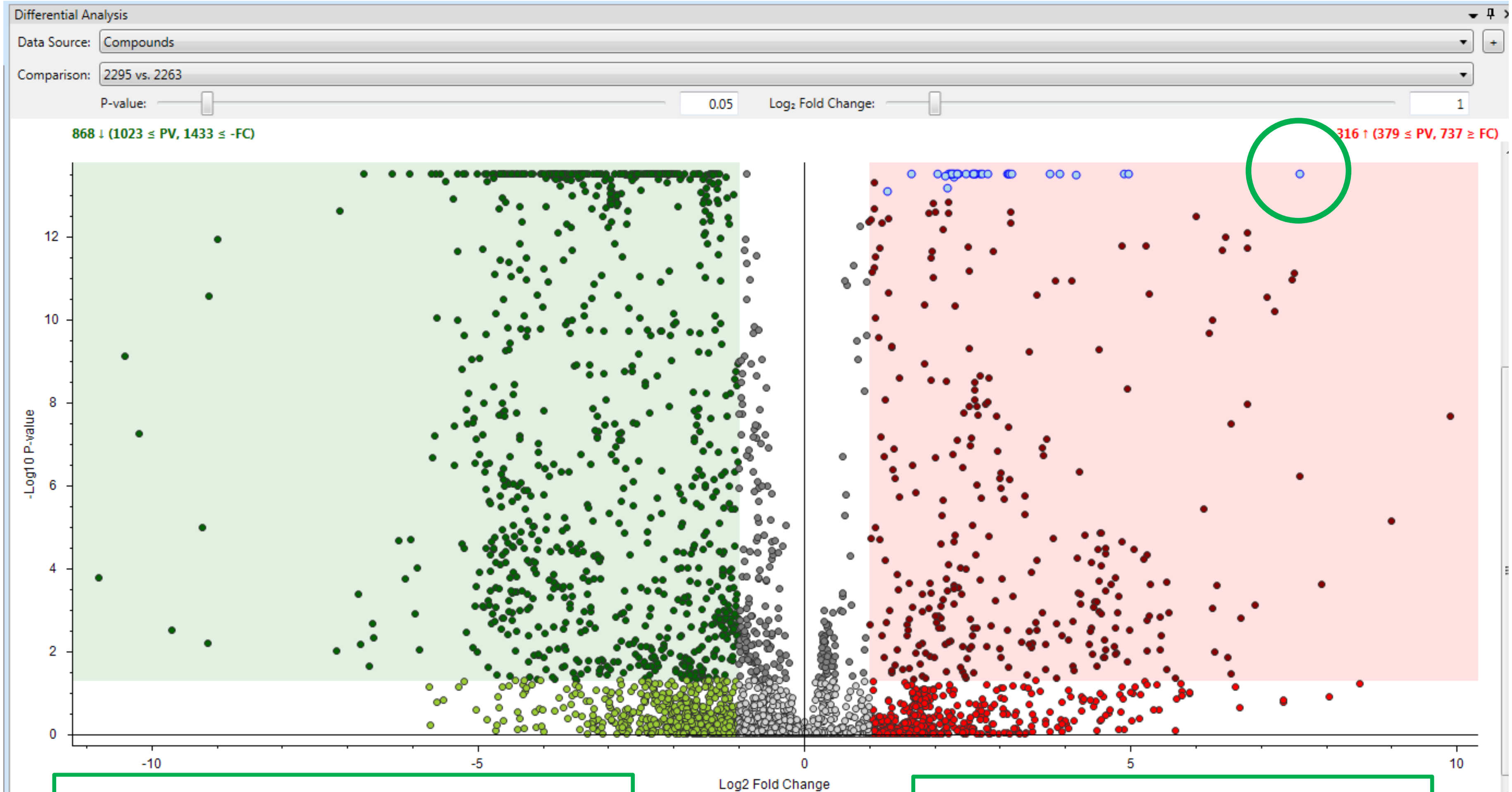
- new charred oak



Single malt aged in 3 Barrels

- **Bourbon**
- **Oloroso**
- **Pedro Ximenez**

Volcano plot between Bourbon and Single whisky



Unique in single malt

Unique in bourbon

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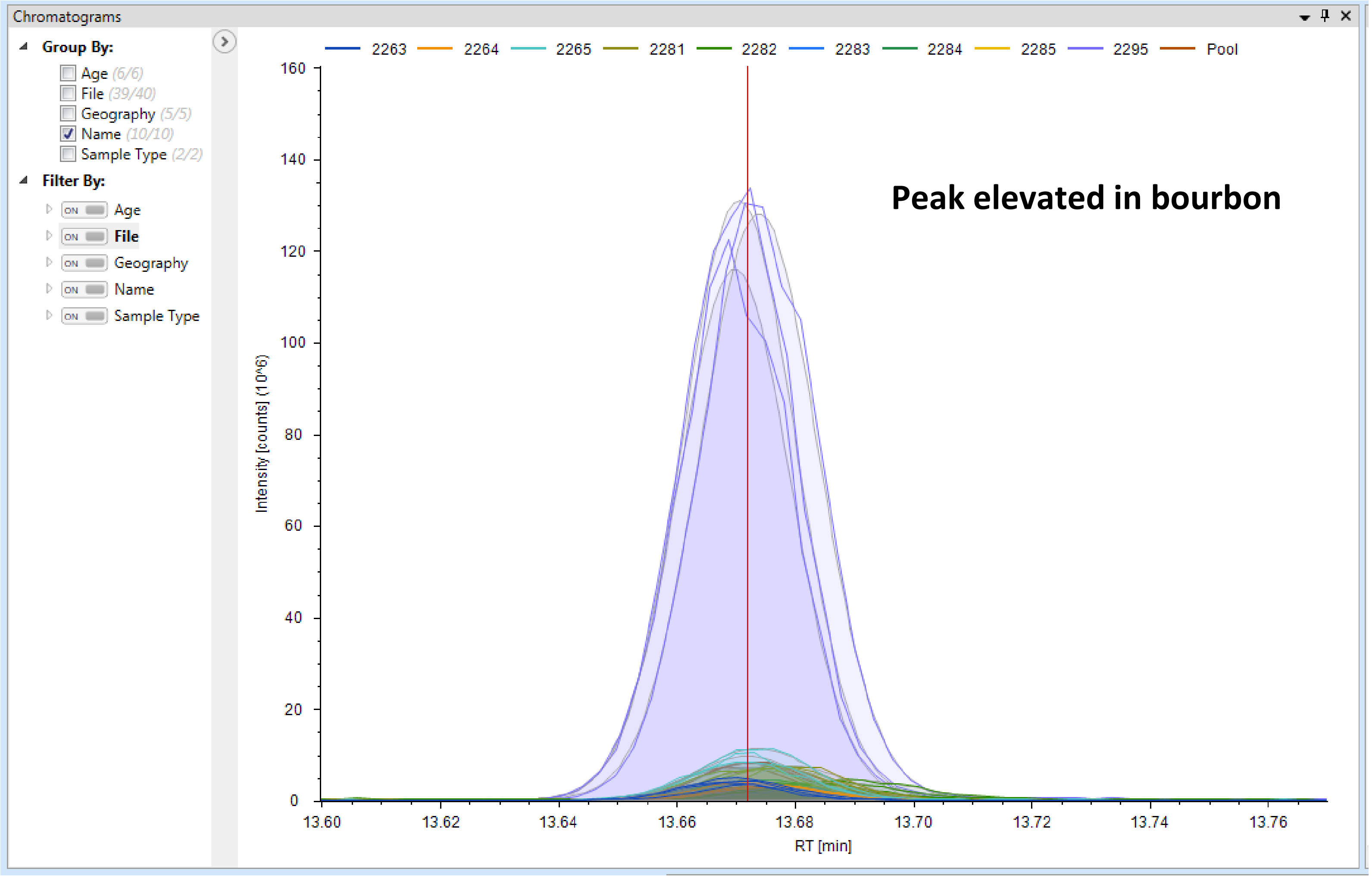
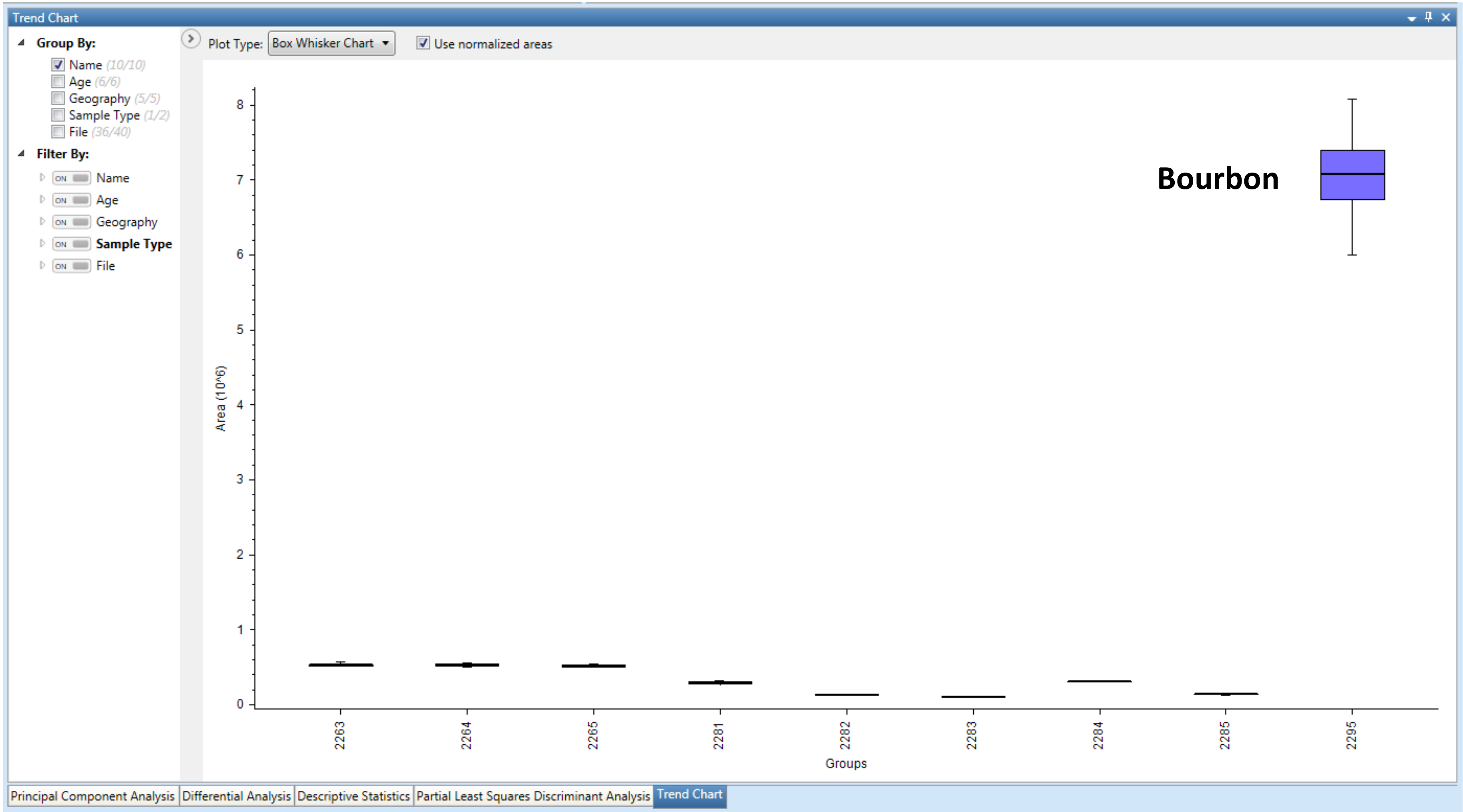
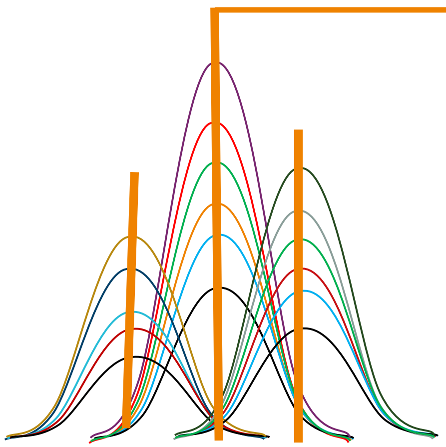
CD 2.1: Extracted ion chromatogram across all samples



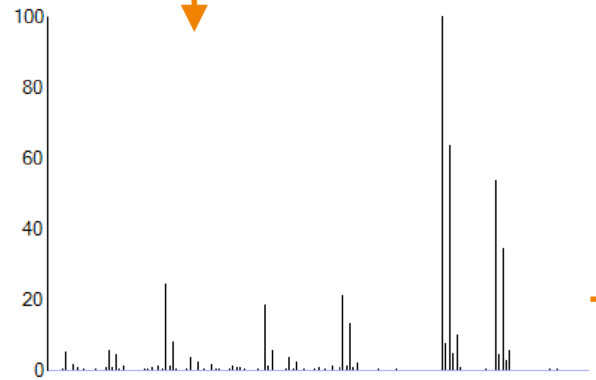
Box Chart of Peak at 13.65 Minutes Across all Samples



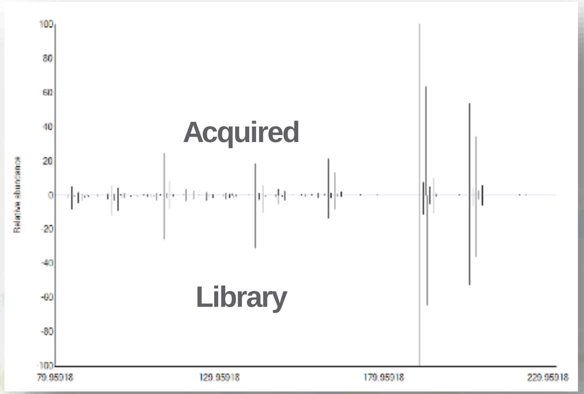
Peak Detection and Candidate Matching



Deconvolve TIC

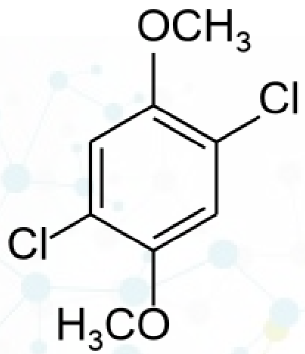


Create “clean” spectrum



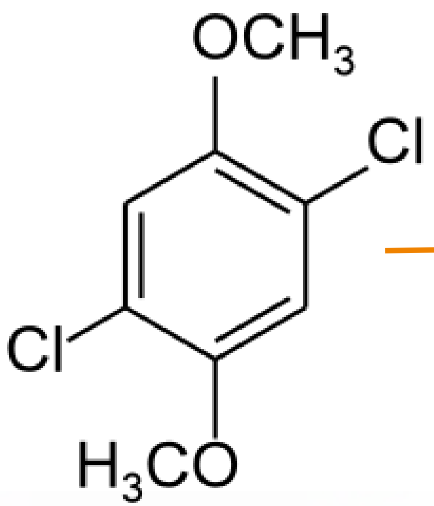
Library search

Candidate Compounds



High Resolution Filtering

Candidate Compounds



$C_8H_8Cl_2O_2$

Subset formulae

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	$C_5Cl_2H_2O$	147.9477	0.20277
148.9369	C_5Cl[37]ClHO	148.9369	0.2679
149.9448	C_5Cl[37]ClH_2O	149.9448	0.06602
151.9419	C_5[37]Cl_2 H_2O	151.9418	0.72528
154.9895	$C_7ClH_4O_2$	154.9894	0.38712
155.9974	$C_7ClH_5O_2$	155.9973	0.89745
157.9943	C_7[37]ClH_5O_2	157.9943	0.25381
159.9479	$C_6Cl_2H_2O$	159.9477	0.87529
161.9446	C_6 Cl[37]ClH_2O	161.9448	0.80213
162.9711	C6Cl_2H_5O	162.9712	0.36816
163.9745	C_5[13]CCl_2H_5O	163.9745	0.3342
164.9682	C_6Cl[37]ClH_5O	164.9682	0.24186
165.9716	C_5CCl[37]ClH_5O	165.9716	0.02832

$$\text{HRF Score} = \frac{\sum (m/z \cdot \text{Intensity})_{explained}}{\sum (m/z \cdot \text{Intensity})_{observed}} \times 100\%$$

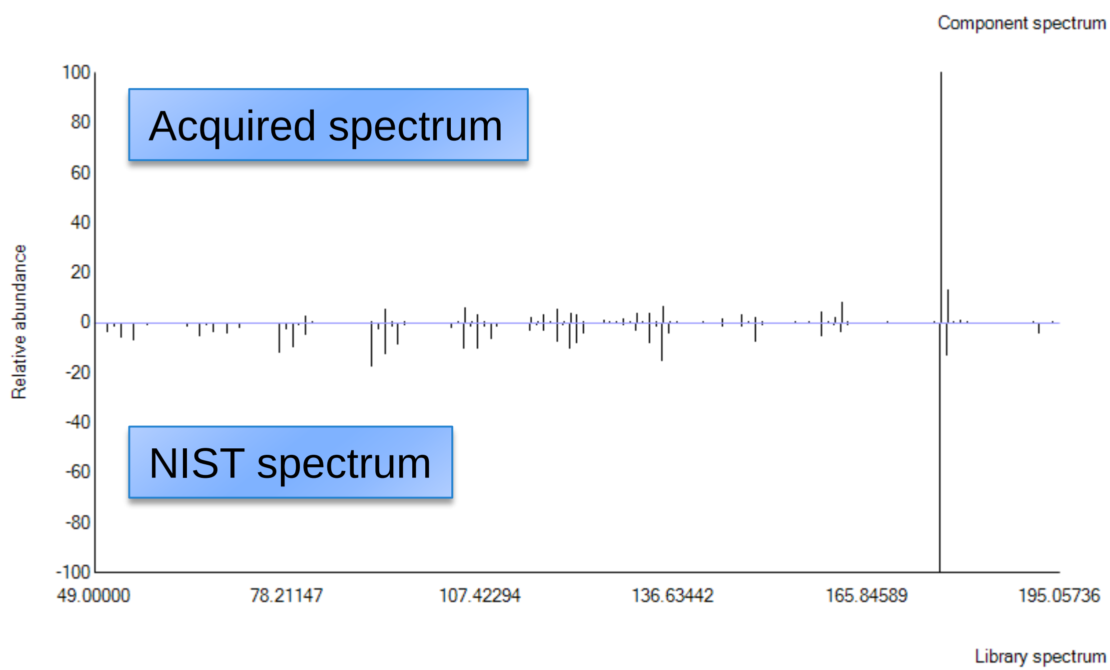
NIST Library Hit for Trans β Ionone

Peak Identification

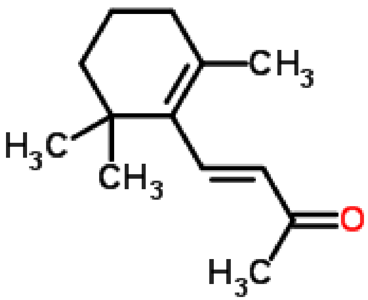
Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	% Elements	Libra
75.3	**trans-β-Ionone**	**C13H20O**	**79-77-6**	**797**	**98.3576**	**192.15086**	**No**	**100**	**mainl**
74.9	3-Buten-2-one, 4-(2,6,6-trim...	C13H20O	14901-0...	776	98.3576	192.15086	No	100	mainl
73.4	Terephthalic acid, ethyl 2-iso...	C19H20O4		673	99.8952	312.13561	No	100	mainl
73.2	5-Methyl-2,4-diisopropylphe...	C13H20O	40625-9...	690	98.3576	192.15086	No	100	mainl
73	Acetic acid, 6,6-dimethyl-2-...	C16H24O4		649	99.8952	280.16691	No	100	mainl
72.9	Isophthalic acid, ethyl tridec-...	C23H32O4		648	99.8952	372.22951	No	100	mainl

NIST hits filtered based on high resolution filtering

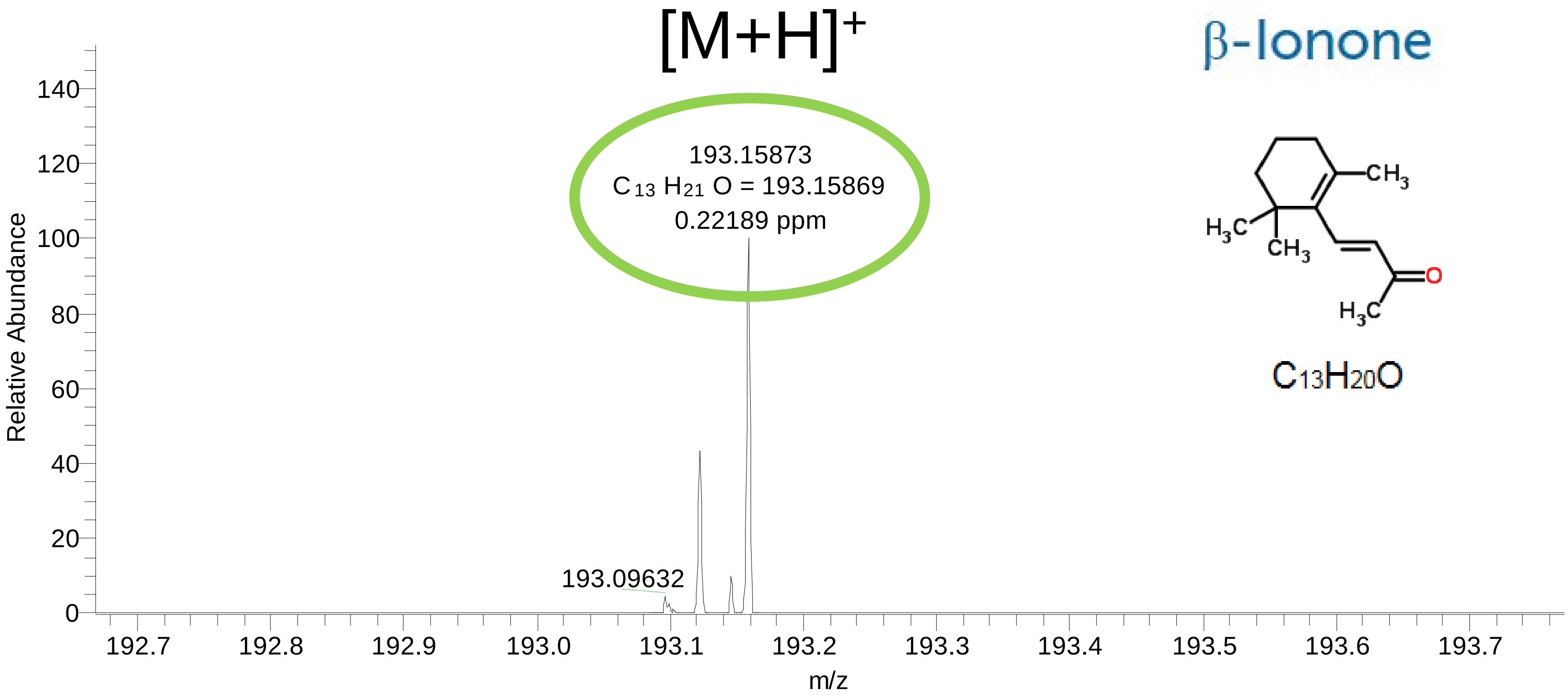
Spectra



Trans β Ionone

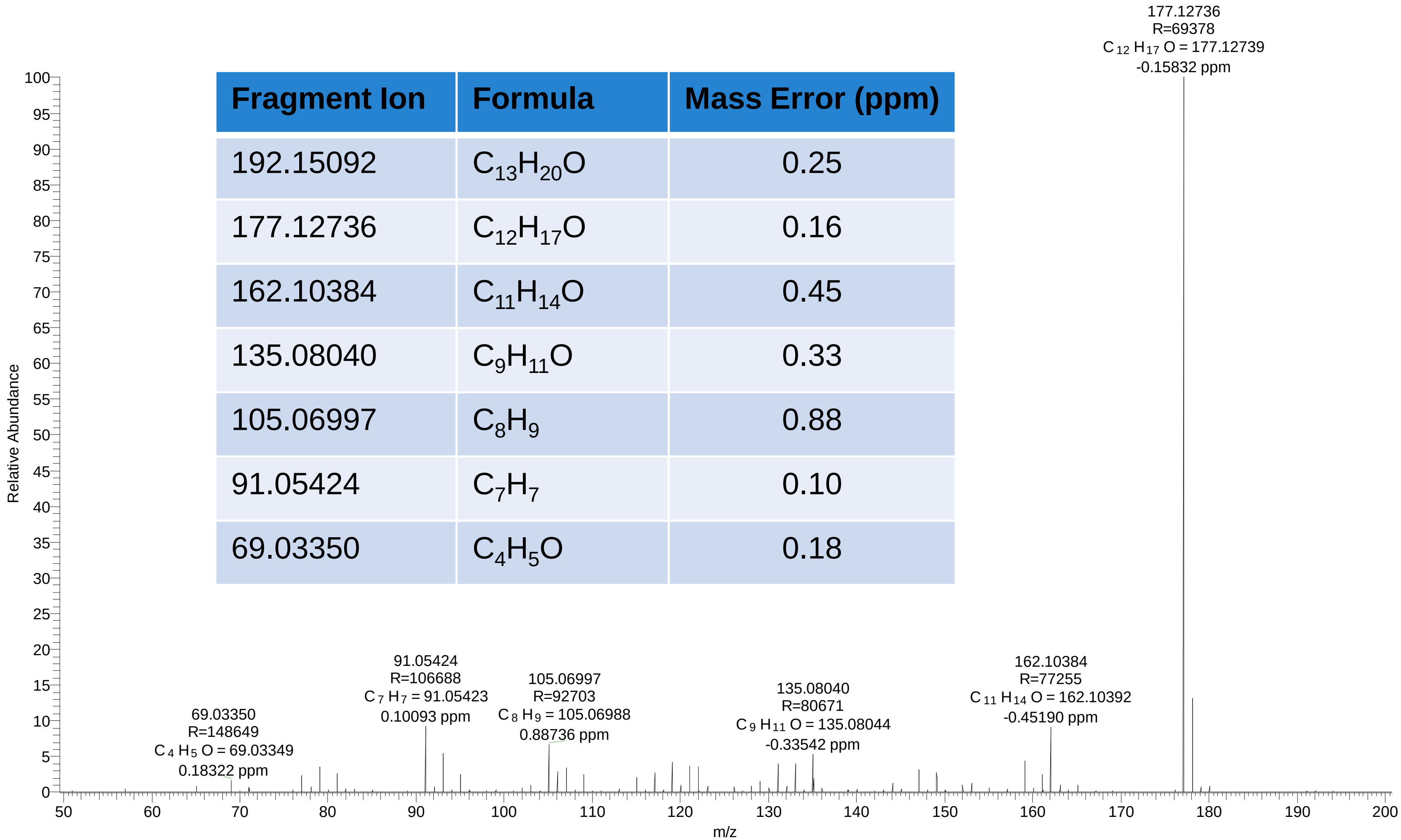


PCI Analysis to Confirm Parent Ion



Parent and 6 Most Intense Fragments Support Identification with <1 ppm

Fragment Ion	Formula	Mass Error (ppm)
192.15092	$C_{13}H_{20}O$	0.25
177.12736	$C_{12}H_{17}O$	0.16
162.10384	$C_{11}H_{14}O$	0.45
135.08040	$C_{9}H_{11}O$	0.33
105.06997	$C_{8}H_{9}$	0.88
91.05424	$C_{7}H_{7}$	0.10
69.03350	$C_{4}H_{5}O$	0.18



Literature Search for Beta Ionone Supports

- Beta-ionone is a breakdown product of beta-carotene and expected to be found in whisky
- Bourbon brewed from corn; Scotch Whisky from barley
- Corn is higher in beta-carotene (53 µg/200g) compared to barley (7 µg/200g (Source: Nutritiondata.com)
- This would support the higher levels of beta-ionone found in our data

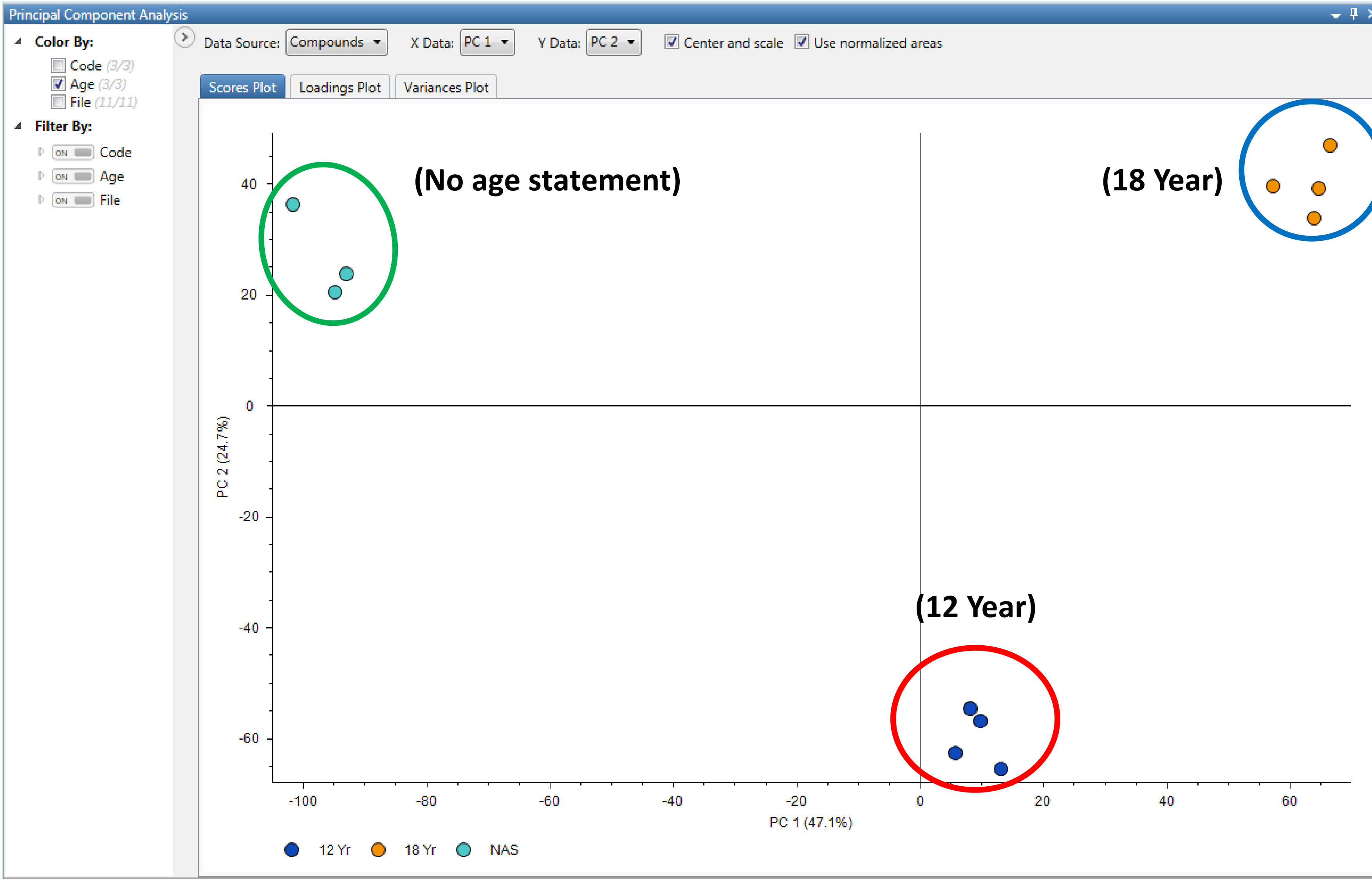
Article

Whiskey composition: formation of alpha-and beta-ionone by the thermal decomposition of beta-carotene

Earl G. LaRoe   Paul A. Shipley

Journal of Agricultural and Food Chemistry (Impact Factor: 3.11). 04/2002; 18(1).
DOI: 10.1021/jf60167a012

PCA for Single Distillery But Different Years



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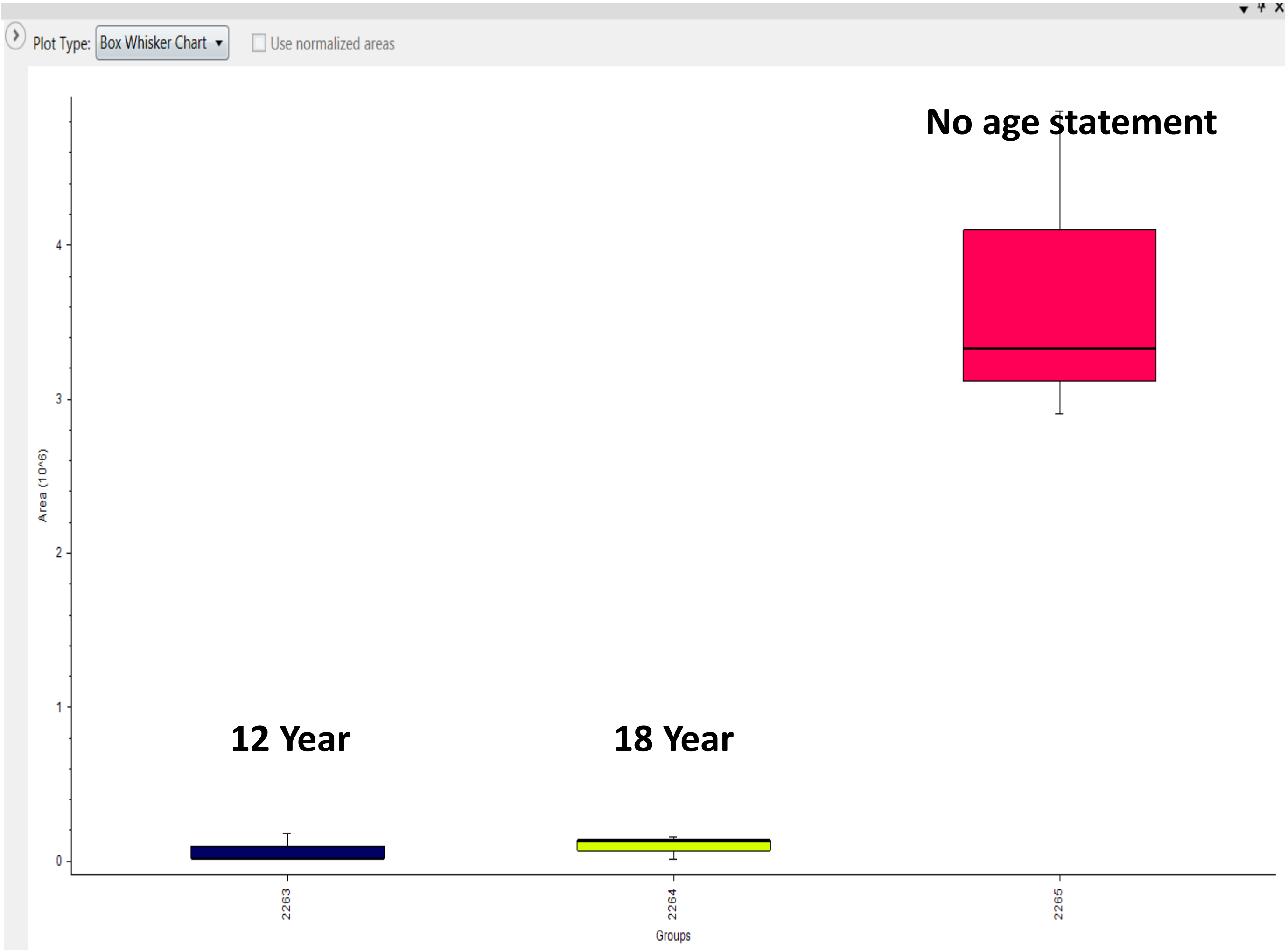
Boxplot of Peak at 10.4 Minutes



Identify the Compound – Searching NIST 14

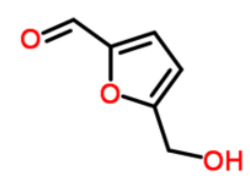
- Identification based on:
    1. Search index (717)
    2. High resolution filtering (HRF) 99.6% of spectrum explained based on $C_6H_6O_3$
    3. Combined score (SI & HRF) 94.2%

- Eliminates other hits that would be valid if only SI used.

Peak Identification

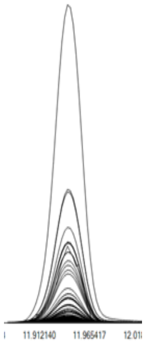
Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	% Elements
94.2	**5-Hydroxymethylfurfural**	**C6H6O3**	**67-47-0**	**717**	**99.6216**	**126.03114**	**Yes**	**100**
69.4	4-Ethyl-2-hydroxycyclopent-...	C7H10O2	28017-6...	708	88.0651	126.06753	No	100
68.5	2-Butyn-1-al diethyl acetal	C8H14O2	2806-97...	662	88.0651	142.09883	No	100
67.9	Cyclopentanecarboxylic acid...	C13H16O2	55229-4...	630	88.1169	204.11448	No	100
67.3	Cyclopentanecarboxylic acid...	C13H22O2		602	88.1169	210.16143	No	100
67.2	Cyclopentanecarboxylic acid...	C12H13NO4		612	99.7754	235.0839	No	75
45.3	4-Hepten-3-one, 4-methyl-	C8H14O	22319-3...	699	28.2184	126.10391	No	100
44.5	4-Hexen-3-one, 4,5-dimethyl-	C8H14O	17325-9...	658	28.2184	126.10391	No	100
44.3	Furan, 2,3-dihydro-4-(1-met...	C8H14O	34379-5...	650	28.2184	126.10391	No	100

Hydroxymethyl furfural

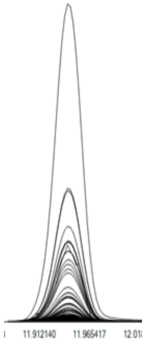


Profiling Samples

- Use the same identification process to build a chemical profile of a sample
- What is in my whisky sample?



= 1037 deconvoluted features



+

= 675 identified compounds

Conclusions

- Q Exactive GC provides a comprehensive chemical profile of a sample, detecting both major and minor components with a high degree of confidence
- In whisky, the chemical differences are rarely unique. However, the concentration of compounds does vary significantly between whiskies of different origins, ages and processes.
- Sophisticated, yet simple to use, software tools provides fast isolation of peaks of interest and intelligent compound identification with sub 1 ppm mass accuracy

Further Details in Application Note 10492 and poster

Application Note 10492

Chemical Profiling and Differential Analysis of Whiskies Using Orbitrap GC-MS

Dominic Roberts,[1] Jana Hajslova,[2] Jana Pulkrabova,[2] and Paul Silcock[1]
[1]Thermo Fisher Scientific, Runcorn, UK
[2]University of Chemistry and Technology, Prague, Czech Republic

Key Words
Chemical profiling, whisky, Q Exactive GC, Orbitrap mass spectrometry, differential and statistical analysis, marker identification, accurate mass

Introduction

Whisky is a premium spirit beverage that is distilled by following long established methods and has a complex aging process. It is produced by the mixing of various grains with water to form a mash that is fermented with yeast, distilled to generate an alcoholic distillate and finally matured in wooden barrels or casks.[1] This is a complex and traditional process that results in a beverage that has both a high value and high degree of variability in the final product depending on many different factors. It is this variability that gives whisky the characteristics that are unique to a particular distillery or region. For example, whiskies produced on the West coast of Scotland often have a very smoky flavor, while those from the Speyside region can have characteristic honey, vanilla and fruit flavors[2]; in general terms, the production technology plays a significant role.

As a result of these distinguishing features and the rising global demand, whisky has become an economically important commodity in many regions of the world. The entire whisky industry is a major source of employment and tax revenues in these regions. For example, the whisky market is worth ~£5 billion to the UK economy,[3] and in the USA, distilled spirits are collectively worth $120 billion.[4] As whisky has a high retail price, counterfeiting and/or adulteration is common and is a threat to the integrity of the industry. The adulteration can take many forms and can occur on both small and large scales. For example, one of the most extensive forms of adulteration is to add the main chemical constituents of whisky to an alternative cheaper spirit to create an "artificial" whisky. This is of particular concern as there is no safety control over which chemicals are being added, their quality or concentration. Other forms of adulteration include the labelling of bottles with more expensive brands and falsely claiming the age for which the whisky was matured in the barrel. The latter type of adulteration can be performed either by the addition of artificial colors or by heating during the aging process to speed up the coloration. Both processes can appear to secure in a few months or days what otherwise would have taken many years to achieve.

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