Identification of Non Volatile Congeners by Accurate Mass LC-TOF to Determine Bourbon Product Authenticity

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Abstract

There are many congeners found in real bourbon as a result of the fermentation, distillation, and the aging process. These congeners make each bourbon unique. Traditionally present in true bourbon are phenols, furans and antioxidants such as: gallic acid, vanillic acid, syringic acid, ellagic acid, syringaldehyde, 5-(hydroxymethyl) furfural, and 2-furaldehyde. The presence and concentration of these congeners is the key in determining if bourbon is genuine. GC and GC/MS are often used for analysis of these compounds, however, these analytes require derivatization for GC analysis. Here we look at a group of non-volatile phenolic compounds and furans, using reverse phase LC with ESI TOF. MassHunter software utilizing Molecular Feature Extractor (MFE) and Molecular Formula Generator (MFG) was used to predict empirical formulae and for compound identification. We were able to identify all 10 compounds via accurate mass, even with co-eluting compounds present.

Introduction

.BY LAW, BOURBON IS...

- *Produced in the USA*
- *Made from a grain mix of at least 51% corn*
- *Distilled at less than 160 proof*
- *Aged in new charred white oak barrels*
- *Aged for a minimum of 2 years*

Jim Beam ages its' bourbon over 4 years in wooden barrels which expand and contract during the changing of the seasons. The mash is absorbed and desorbed into the gator charred oak of the barrel. From this process, natural caramelized sugars add color and additional flavors to the bourbon. Over 1 million dollars in revenue is lost yearly from sales of counterfeit bourbon.

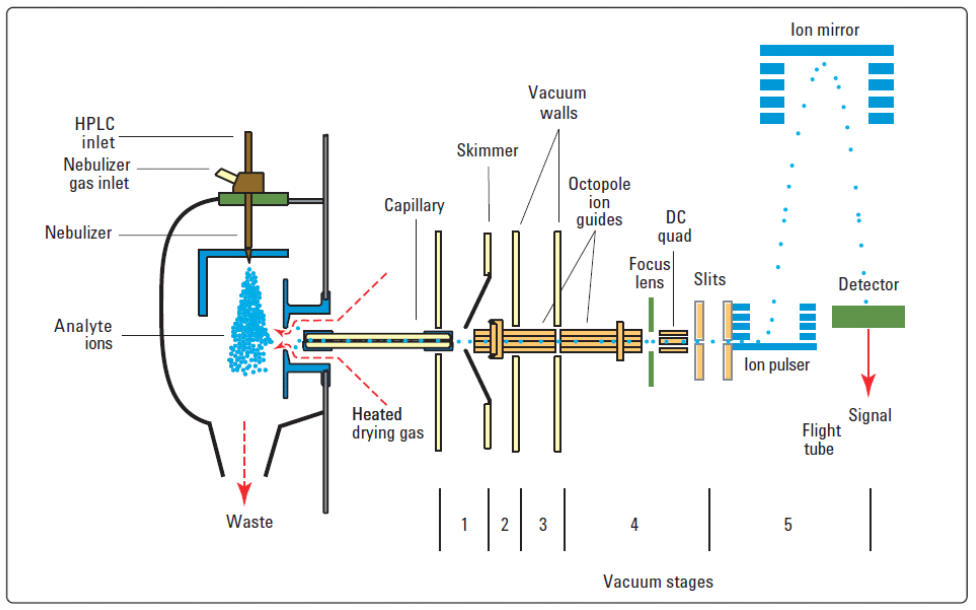
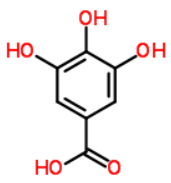


Diagram of the LC/MS-TOF

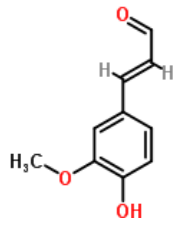
Non-Volatile Congeners Structures

Gallic Acid



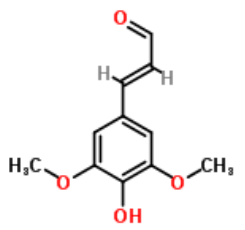
$C_7H_6O_5$
170.0215

Coniferaldehyde



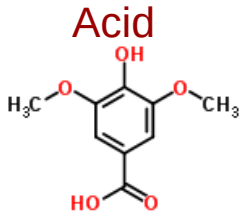
$C_{10}H_{10}O_3$
178.0630

Sinapaldehyde



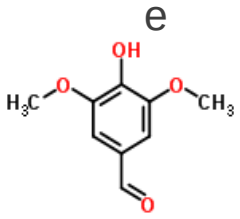
$C_{11}H_{12}O_4$
208.0736

Syringic Acid



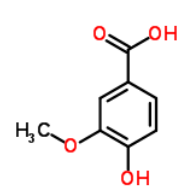
$C_9H_{10}O_4$
182.0579

Syringaldehyde



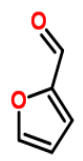
$C_9H_{10}O_5$
198.0528

Vanillic Acid



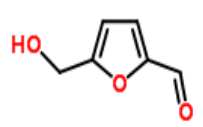
$C_8H_8O_4$
168.0423

Furfural



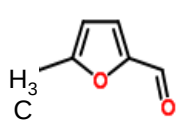
$C_5H_4O_2$
96.0211

5-HMF



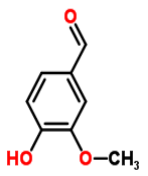
$C_6H_6O_3$
126.0317

5-



$C_6H_6O_2$
110.0368

Vanillin



$C_8H_8O_3$
152.0473

List of Abbreviations

- *TOF - Time of Flight*
- *MS - Mass Spectrometer*
- *ESI - Electro Spray Ionization*
- *TIC - Total Ion Chromatogram*
- *EIC - Extracted Ion Chromatogram*
- *DAD - Diode Array Detector*
- *UV - Ultra Violet*
- *MFE - Molecular Feature Extractor*
- *MFG - Molecular Formula Generator*

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Experimental

HPLC Conditions

Agilent 1200 RR-HPLC series Binary Pump SL, Well Plate Sampler SL, Thermostatic Column Compartment SL, DAD-SL

Column: Zorbax SB C18, 2.1 x 100mm, 1.8 µm

Zorbax Eclipse Plus, 2.1 x 100mm, 1.8 µm

Column temperature: 35°C
Injection volume: 0.5 µL
Autosampler temp: ambient ºC
Needle wash: 10 s Flush Port (25:25:50) (H_2O:IPA:MeOH)
DAD-UV 254 nm

Mobile phase: A = 0.1% Formic Acid in Water
B = 0.1% Formic Acid in Methanol
Flow rate: 0.3 mL/min
Gradient:

Time (min)	%B
0.0	5
0.7	95

Stop time: 12.8 min.
Post time: 5.0 min.
Overall run time 17.8 minutes (incl. re-equilibration)

MS: Agilent 6230A Time of Flight Mass Spectrometer

TOF MS Parameters

Ion Mode	Dual ESI,
PositiveMass Range	90-1200 m/z
Scan Rate	2 Hz
Reference Masses	On (121.0509, 922.0098)

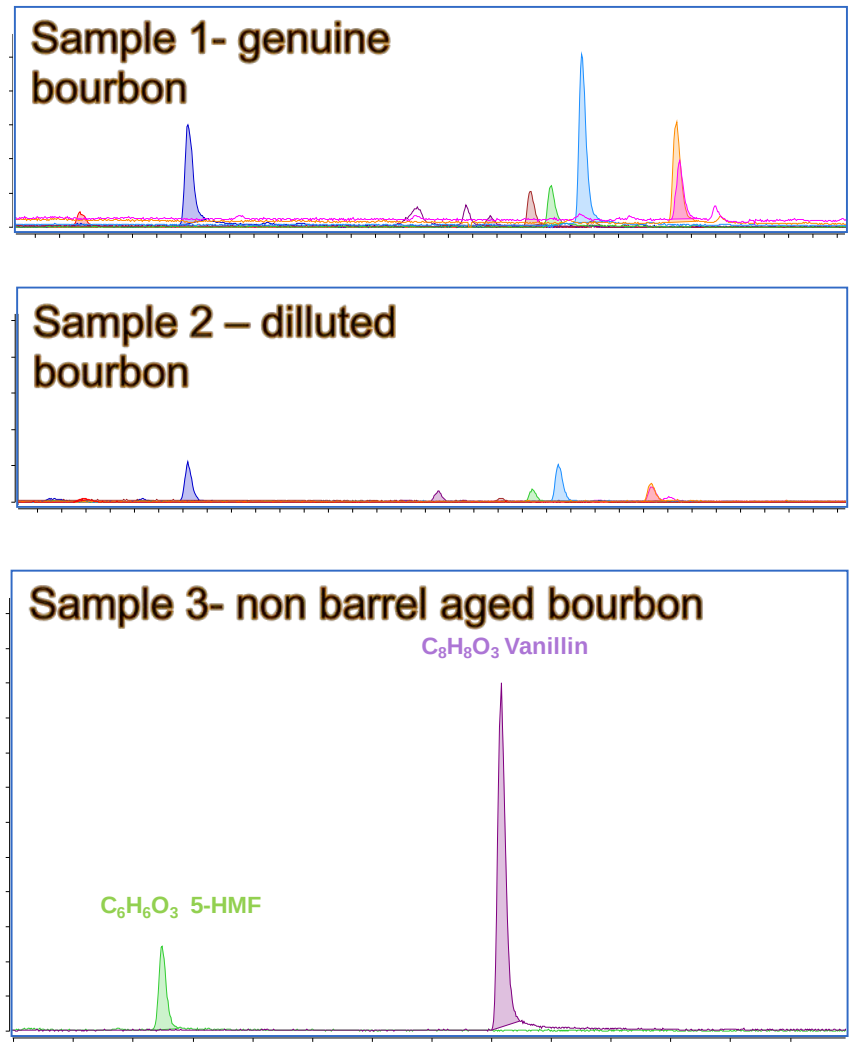
Source Parameters

Drying gas (Nitrogen)	10 L/min
Drying gas temperature	300 ºC
Nebulizer gas (Nitrogen)	40 PSI

Scan Source Parameters

Capillary Voltage	4000 V
Fragmentor	145 V
Skimmer 1	65 V
Octopole RF Peak	750 V

Results and Discussion



The MassHunter Molecular Feature Extractor (MFE) and the Molecular Formula Generator (MFG) unequivocally validate the presence or absence of the key congeners in suspected bourbons.

Sample 2 and 3 exemplify two of the most common counterfeiting techniques - dilution and coloration to fake true barrel aging. UV detection was also utilized for additional verification.

Conclusions

LC/MS TOF enabled us to easily and accurately determine the presence of key non-volatile congeners in both authentic and counterfeit bourbon. With the accurate mass of 1 ppm or better and MassHunter software, we were able to generate empirical formulae for all congener standards. We could then automate the analysis and quickly analyze and report the authenticity of the "suspected" counterfeit samples that were submitted.

Analysis time was less than 9 minutes with no sample preparation. This represents significant time savings when compared to GC/MS analysis which requires derivatization for these types of samples.

Additional work for the quantitation of these samples has not been included in the poster. Today's TOF technology can produce quantitative as well as great qualitative data. Analyzing the pesticides in bourbon is the next step in this ongoing project

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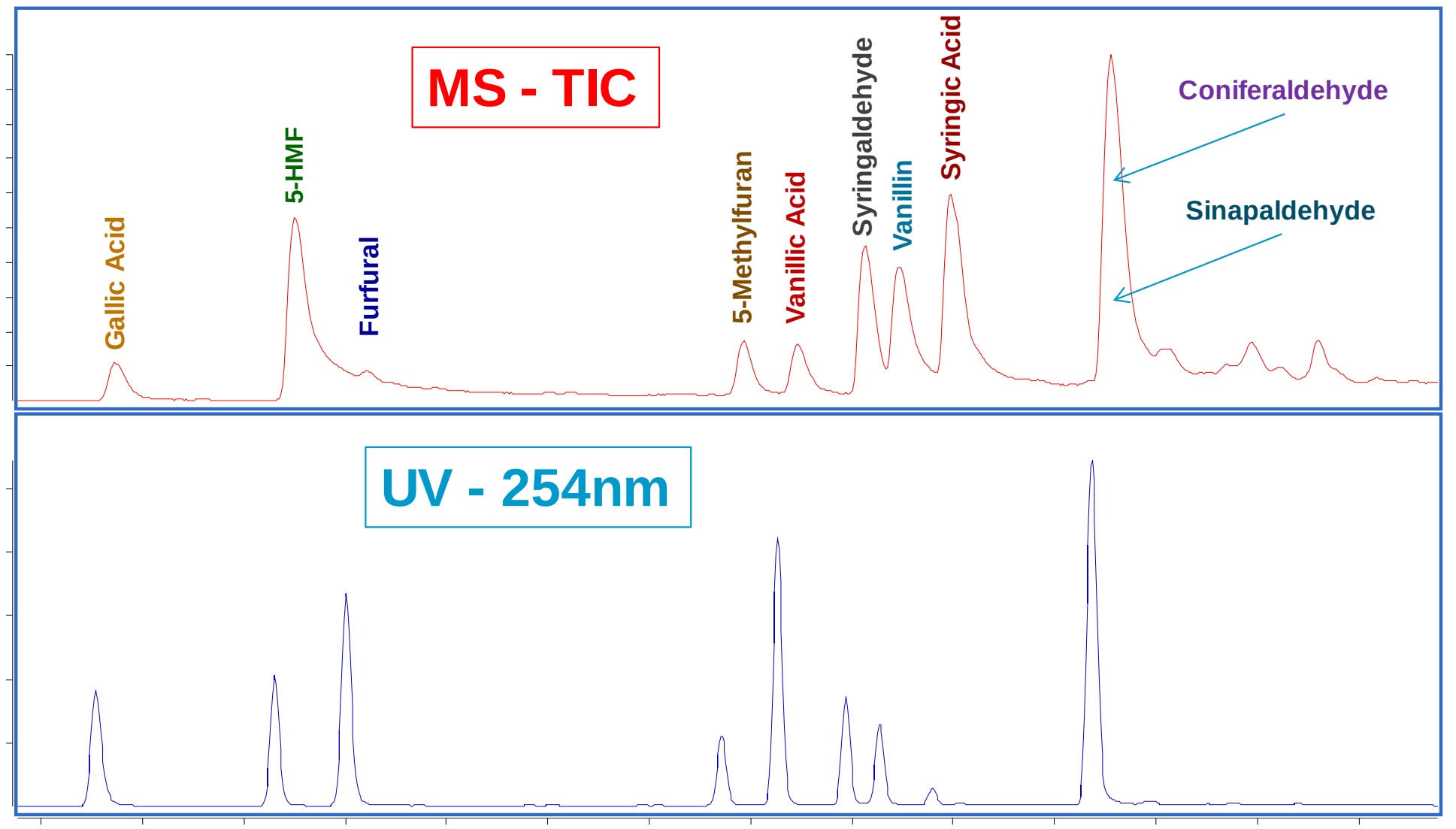
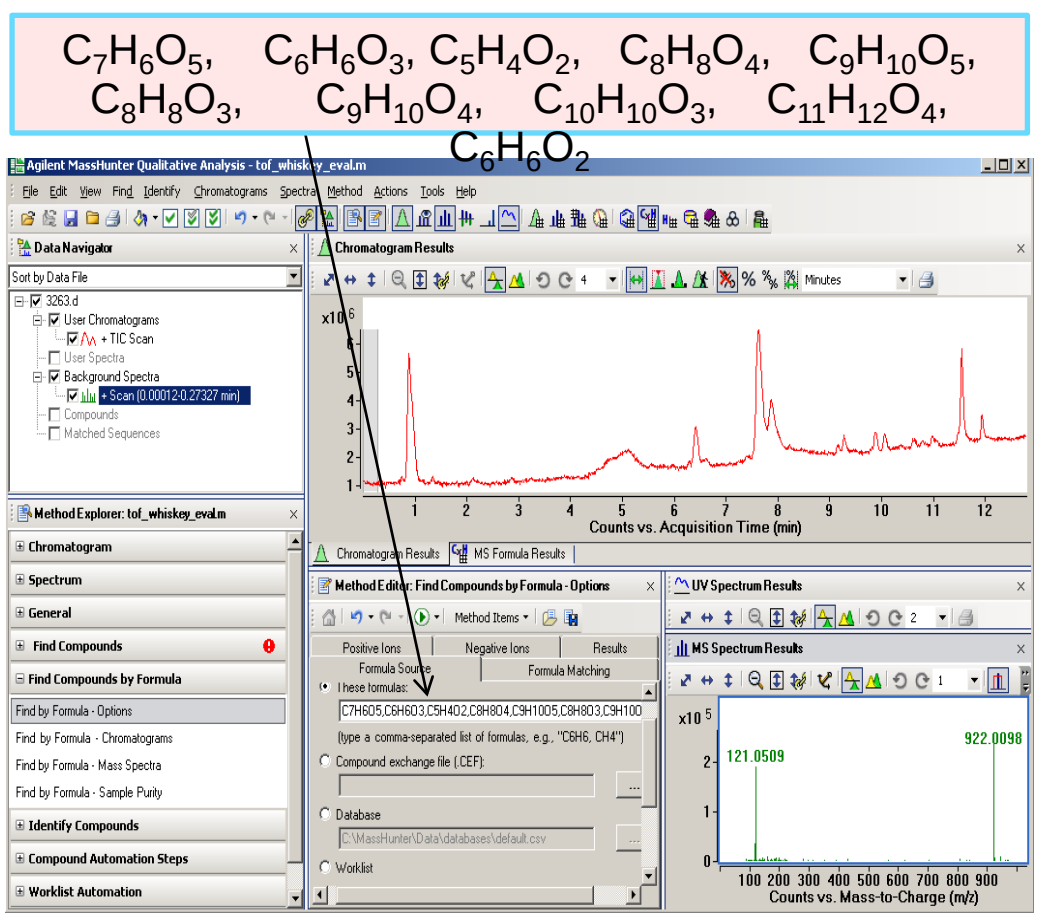
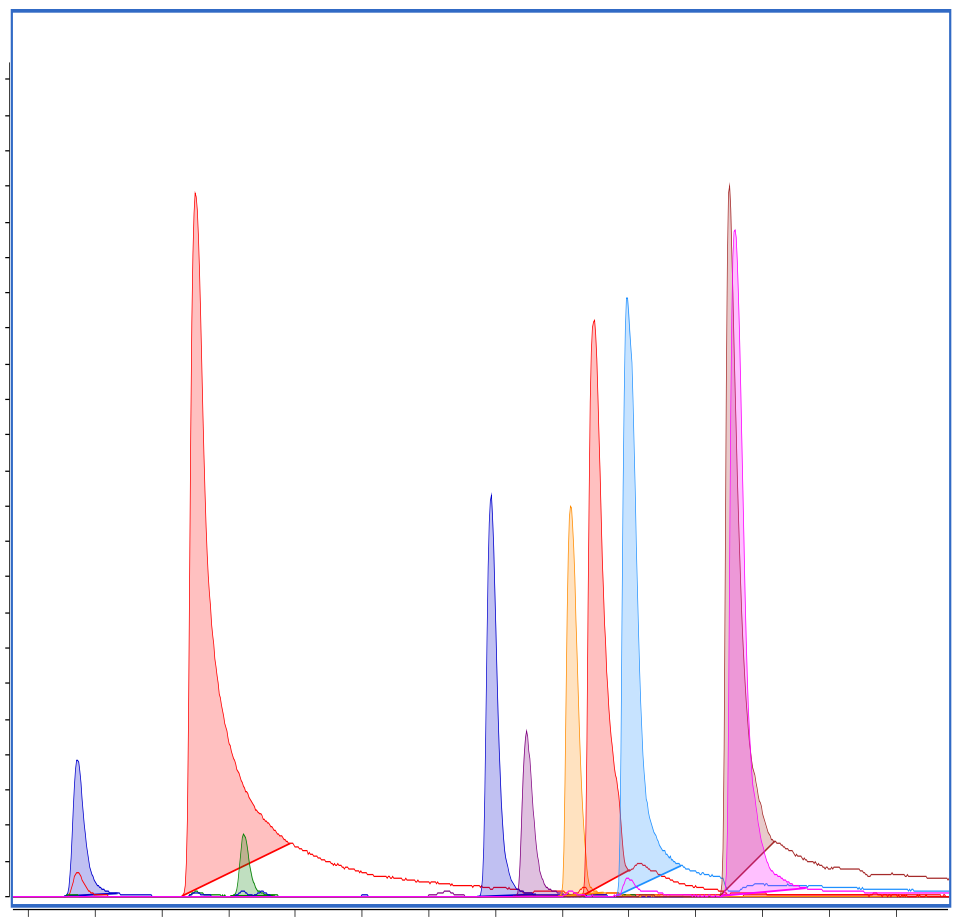


TIC and UV of the 10 Non Volatile congeners in the Standard Mix

$C_7H_6O_5$, $C_6H_6O_3$, $C_5H_4O_2$, $C_8H_8O_4$, $C_9H_{10}O_5$, $C_8H_8O_3$, $C_9H_{10}O_4$, $C_{10}H_{10}O_3$, $C_{11}H_{12}O_4$, $C_6H_6O_2$





Results of the Mass Hunter search algorithm for the10 Non- Volatile congeners in the Standard Mix