Headspace-Solid Phase Micro Extraction – Gas Chromatography – Mass Spectrometry (HS-SPME-GC-MS) Analysis for Oak Barrel Flavor Compounds in Whiskey

By

Hailey Frances Jarzynka

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Kevin A. Schug, Supervising Professor

Daniel Armstrong

Junha Jeon

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Hailey Jarzynka, Hailee E. Anderson[1], Robert H. Magnuson II[1], Tiffany Liden[1], Colton Myers[2]
Robert Arnold[3], Kevin A. Schug[1*]

AUTHOR INFORMATION:

1. Department of Chemistry and Biochemistry, The University of Texas at Arlington, 700 Planetarium Place, Arlington, TX 76019, USA
2. Restek Corporation, Bellefonte, PA
3. Firestone and Robertson Distilling Co., 4250 Mitchell Blvd, Fort Worth, TX 76119, USA

*Correspondence to: Dr. Kevin Schug, 700 Planetarium Pl., Box 19065, Arlington, TX 76019-006;

(email) kschug@uta.edu; (phone) (817)-201-7680

Abbreviations

DVB	Divinylbenzene
ESI	Electrospray ionization
GC	Gas Chromatography
HS	Headspace
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantitation
MeOH	Methanol
MS	Mass spectrometry
MRM	Multiple reaction monitoring
PDMS	Polydimethylsiloxane
QC	Quality control
RSD	Relative standard deviation
RT	Retention time
SPME	Solid phase microextraction
TCA	2,4,6-trichloroanisole
Geo	Geosmin
4ep	4-ethylphenol
4eg	4-ethylguaiacol
Eu	eugenol
V	vanillin
2m4mp	2-methoxy-4-methylphenol
5mf	5-methylfurfural
Gu	guaiacol
Oak	trans-oak lactone
2m4vp	2-methoxy-4-vinylphenol

3	Jim Beam Bonded
4	Old Crow
6	Jim Beam Black Extra Aged Bourbon
8	Jim Beam Kentucky Straight Bourbon Whiskey
41	Old Grad-Dad Bonded
21	Jim Beam Red Stag
228	For Peat's Sake

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1.1 Abstract

This study presents a method to simultaneously detect both fault compounds and oak barrel flavor compounds in whiskey using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). A major contaminant in whiskey is geosmin and an array of oak barrel compounds includes 2-methoxy-4-methylphenol, 5-methylfurfural, guaiacol, trans-whiskey lactone, 2-methoxy-4-vinylphenol, 4-ethylguaiacol, eugenol, vanillin, and 4-ethylphenol. Geosmin is a fault compound that produces a musty or moldy taste to the whiskey and the other compounds originate from the barrels in which the whiskey is aged in and transfer to the whiskey in the aging process.

Each compound was quantitated and 15 different whiskies were compared to one another in the MRM mode. The whiskies were further compared using the SIM mode to compare the areas on the compounds on interest to all the other components in the whiskey.

1.2 Introduction

Dating back over one thousand years, with the debatable origin of Ireland or Scotland, whiskey has been a beverage that many enjoy. Whiskey emerged from these two countries due to monasteries fermenting and distilling a grain mash in the absence of grapes. Hence, whiskey as we know it was created. The general process of the production and distillation of whiskey is relatively similar across all fronts. The process of whiskey making consists of preparing grains, mashing boiled grains and letting it cool, adding yeast for fermentation in barrels, then grains are separated and distilled before being put in oak barrels for aging, finally poured into glass bottles. As the whiskey ages in barrels, a certain amount of transference from barrel to beverage.

The process of maturing beverages such as wine, or whiskey in this case, dates back to the ancient Egyptians. Throughout this maturation and aging process, aromatic substances that enrich the beverage are extracted from the wood. In addition to contributing to flavors and aromatics, aging beverages such as wine or whiskey in wood barrels assist in the process of enriching and stabilizing the color of the beverage.[1] Previous studies have shown that re-using barrels for this aging process yield a lesser quality due to lower concentrations of aromatic components in the used barrel than in a new barrel.[2]

Yeast plays a large role throughout the fermentation and maturation process for both wine and whiskey. Yeast contributes to a process called malolactic fermentation, which in turn assists in the release of vanillin. The lactic acid that is produced during the malolactic fermentation converts a precursor of vanillin into its final product, greatly contributing to the aromatics found in wines and whiskies.[3]

In addition to contributing to flavor and aroma components of wine and whiskey, oak barrels serve several other beneficial purposes. Firstly, oak contains tyloses and ellagitannins, which are vital for fungal degradation resistance, making oak barrels desirable for the aging process. Secondly, oak barrels contain polyphenols, which are released in abundance from the wood, contributing to a greater abundance of antioxidants within these beverages.[4]

Quality control of flavor components has dominated in the wine industry – since wine is aged in barrels. Some of the most important sensory components that come from aging in oak barrels include vanillin and eugenol. These two flavors contribute green wood and spicy aromas to the alcoholic beverage, respectively. There are several other important flavor components that are present in the wood and contribute to the flavors of alcoholic beverages, including guaiacol, which gives a smoky aroma, furfural compounds, which provide a hint of almond, and lactones, in this case, whiskey lactones, which contribute the woody and coconut aroma.[1]

There have also been several advancements in a technique called micro-oxygenation, in which small amounts of oxygen are released, assisting in enhancing the aging process of the wine or whiskey within the oak barrel. Even when this technique is not used, proper oxygenation of yeast allows for the aromatic modifications within the beverage, as well as removing any flavor components that are undesirable. Within winemaking, oxidation can become an issue, so it is also vital to stop excessive oxidation, which could lead to undesired microbials or microorganisms that could spoil the beverage. With wine in particular, sulfur dioxide is added to prevent this excessive oxidation, but sulfur dioxide is highly undesirable in whiskey.[1] With whiskey, the best way to oxidation is proper bottling, including keeping the cork

moist to prevent crumbling, which would allow oxidation, as well as properly sealing the bottle or transferring the whiskey into a smaller bottle once opened.

In addition to flavor compounds that mostly come from transference of the oak barrel compounds, taint compounds can give unpleasant flavors to the beverage. Geosmin is a fault compound that has a musty and unpleasant taste and smell. Geosmin can be introduced to a beverage though the water used in production, moist grain, or transference from the barrel.[5–10]

SPME Arrow is a solvent free sample preparation that boosts productivity when compared to classic SPME fibers. The SPME Arrow provides a higher sensitivity up to ten times higher than traditional SPME fibers.[11] The extraction time is faster and has improved robustness and can be used with a wide range of sorption materials. The SPME arrow fibers are also conveniently color-coded based on their phase and diameter. SPME arrow is also a much cleaner application for volatiles when compared to the direct injection method that is commonly used.

In this study, we aim develop a method to simultaneously analyze an off-flavor, geosmin, and several oak volatiles, namely 2-methoxy-4-methylphenol, 5-methylfurfural, guaiacol, trans-whiskey lactone, 2-methoxy-4-vinylphenol, 4-ethylguaiacol, eugenol, vanillin, and 4-ethylphenol in whiskey for routine quality control using HS-SPME-GC-MS.

Analyses were conducted using a Shimadzu GCMS-TQ8030 gas chromatograph – triple quadrupole mass spectrometer with an AOC-6000 autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) for automated headspace SPME extraction and desorption. The Triple Quadrupole MS is the most common MS/MS system. This type of system when used

can provide great selectivity, sensitivity and also specificity when you use precursor scans and MRM (multiple reaction monitoring).[12] This occurs when there is an ion source. The first and third quadrupoles act as mass filters while the second quadrupole uses the collision gas in the collision cell for fragmentation of the precursor ion or analytes. The first quadrupole is used for the selection of the precursor ion and the third quadrupole is used for the selection of the fragment ions.

1.3 Materials and Methods

1.3.1. Chemicals and Reagents

Standards of 2-methoxy-4-methylphenol, 5-methylfurfural, guaiacol, trans-whiskey lactone, 2-methoxy-4-vinylphenol, 4-ethylguaiacol, eugenol, vanillin, and 4-ethylphenol were purchased from Sigma Aldrich (St. Louis, MO, USA) to analyze for oak barrel flavor components. Geosmin (100 mg/L in methanol) from Sigma Aldrich (St. Louis, MO, USA) were observed to identify possible "off" flavors. LCMS grade methanol was obtained from Honeywell (Muskegon, MI, USA). Sodium chloride (certified ACS) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ethanol (200 proof) was purchased from Decon Laboratories, Inc. (King of Prussia, PA). LCMS grade water was purchased from Avantor Performance Materials, LLC (Radnor, PA, USA). Several varieties of whiskey were selected from a local liquor store (USA).

1.3.2. Method Validation

Fresh stock solutions of 1000 mg/L were prepared in methanol for all compounds. Working solutions of 100 mg/L were prepared in methanol and the subsequent dilution to 1 mg/mL, 100 µg/L and 1 µg/L were made in LCMS grade water.

Calibration curves were created for each analyte of interest. 20 mL headspace (HS) vials (Restek Corporation, Bellefonte, PA) were used to prepare each point for calibration, containing 3 grams of sodium chloride, each analyte of interest, and diluted to 5 mL with LCMS water and 5% ethanol. Ethanol (200 proof) was used for the calibration of the oak barrel components and a highly filtered whiskey was used for the 5% ethanol for the "off" flavors. Limit of detection (LOD) and the correlation factor (R^2) were calculated using the calibration data. Each calibration level point was measured in triplicate.

1.3.3. Samples

Samples of whiskey were obtained from a local liquor store were prepared by diluting with LCMS water to 5% ABV (alcohol by volume) in a headspace vial with 3 grams of sodium chloride. Each whiskey sample was analyzed in triplicate, with results being reported at 40 proof to provide a direct comparison.

1.3.4. Instrumentation

The flavor compounds and fault compounds of interest were analyzed simultaneously. Analyses were conducted using a Shimadzu GCMS-TQ8030 gas chromatograph – triple quadrupole mass spectrometer with an AOC-6000 autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) for automated headspace SPME extraction and desorption. SPME Arrow with a diameter of 1.10 mm and a divinylbenzene/polydimethylsiloxane (DVB/PDMS) fiber (film thickness: 120 µm) was used to extract the volatiles from the headspace. Restek Corporation (Bellefonte, PA) supplied the SPEME Arrow. The HS-SPME temperature programming was to use a 40 °C extraction temperature, an extraction time of 10 minutes, an incubation time of 2 minutes, and a 1 minute desorption time.

Separation was achieved using a mid-polarity, Rxi-624Sil MS column (30 m x 1.4 µm x 0.25 mm) from Restek. Helium was used as the carrier gas, with a flow rate of 1.69 mL/min and a linear velocity of 47.2 cm/sec. The injection temperature was set to 280°C. Splitless injection was used during desorption; the split vent was opened to a split ratio of 5:1 after a 1 minute hold time in the splitless mode. The temperature program started at 50 °C (hold for 3 minutes), ramped at a rate of 8 °C/min to 180 °C (hold for 11 minutes), and then ramped at a rate of 25 °C/min to 280 °C (hold for 5 minutes).

The ion source temperature of the mass spectrometer was set to 230 °C. The analytes were measured in MRM mode (multiple reaction monitoring) using the transitions listed in Table 1. The precursor ion was selected based on the highest abundance fragment observed in the ionization of each compound. A product ion scan was conducted to observe the fragmentation of the precursor ion to identify the product ions. One product ion chosen is for quantitation and the other is for qualitative identification.

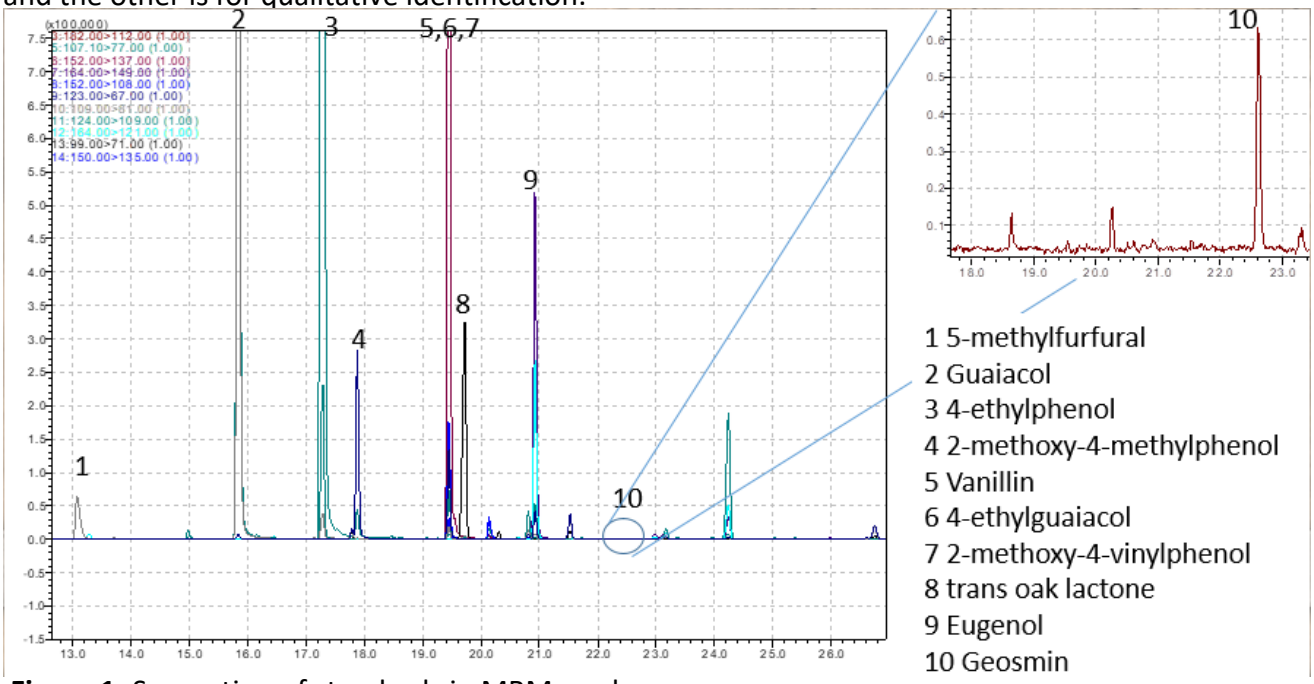


Figure 1: Separation of standards in MRM mode

Table 1: List of the analytes used in this study, their MRM transitions, retention times and collision energy.

Analyte	Precursor ion	Product ion 1 (Quant)	Product ion 2 (Qual)	Retention Time (min)	Collision Energy (V)
Geosmin	182	112	125	22.60	15
2-methoxy-4-methylphenol	123	67	55	17.90	14
5-methylfurfural	109	53	81	13.10	16
Guaiacol	124	109	81	15.85	14
trans-oak lactone	99	71	53	19.70	14
2-methoxy-4-vinylphenol	150	135	107	19.50	15
4-ethylguaiacol	152	137	122	19.45	14
Eugenol	164	149	104	20.90	21
Vanillin	152	108	52	19.45	14
4-ethylphenol	107	77	51	17.25	16

1.4 Results and Discussion

1.4.1. SPME Optimization

Previously conducted optimization studies were centered on geosmin, since the detection of fault compounds take priority over quantification of flavor components from the oak barrel. Spiked whiskey samples with 450 ng/L geosmin was used. The alcohol content, addition of salt, extraction and adsorption times were tested by changing a single variable each run.

The alcohol content for whiskey sample preparation were tested at concentrations of 20, 10 and 5% by volume. Each whiskey was diluted according to its reported ABV or proof. The effect of the alcohol content was observed for both the SPME fiber and geosmin. Matrix

effects were observed at 20% alcohol and 5% alcohol provided the highest response as shown in Figure 1.

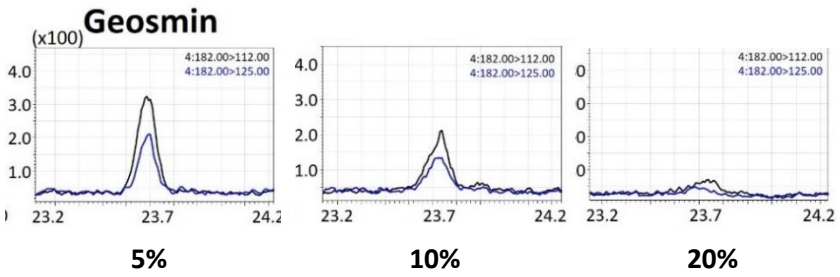


Figure 2: Effect of alcohol content on geosmin response when spiked (450 ng/L) into a whiskey sample. The MRMs for the geosmin are on the same scale.

An inorganic salt is often added to the sample to increase the concentration of volatiles in the headspace.[1] Sodium chloride (NaCl) addition of 1.5 grams, 3 grams, and 6 grams were investigated. The addition of 3 grams NaCl resulted in the highest recovery of the analytes.

SPME extraction times of 4 min, 10 min, 15 min, and 20 mins, and desorption times of 10 s, 1 min, 3 min, and 6 mins were also evaluated. 4 min was not long enough for geosmin extraction and there were no significant differences in extraction efficiency between 10, 15, or 20 min. 10 mins was used as the extraction time to increase analysis speed. In addition, 1 min was chosen as the best desorption time for efficiency.

1.4.2. Quantitation and Method Validation

Calibration curves were created for each compound. An internal standard of 3-chlorotluene was evaluated, spiked at 500 ng/L. but did not improve linearity of the calibration curves, so the value was not used. Each curve contained a minimum of 7 calibration levels and were run in triplicate. The concentrations selected for calibration were determined based on the expected span observed in the whiskies when diluted to 5% alcohol.

Table 2: Calibration curve information for the targeted analytes, including LOD, LOQ, correlation factor, % RSD, accuracies (% error) and precision (CV). Concentrations were dependent on the LOD

Analyte	**LOD**	**LOQ**	**R^2**	**Accuracy** **L | H**	**Precision** **L | H**	**%RSD**
Geosmin	50 ppt	165 ppt	0.9972	2.3 | 6.4	1.6 | 22.4	16.6 %
2-methoxy-4-methylphenol	2 ppb	10 ppb	0.9645	146 | 24.9	0.8 | 19.5	14.0 %
5-methylfurfural	5 ppb	2 ppb	0.9991	17.7 | 2.6	0.08| 14.5	9.6 %
Guaiacol	1.5 ppb	1.5 ppb	0.9974	15.2 | 1.3	0.4 | 3.0	2.4 %
trans-oak lactone	2 ppb	7.5 ppb	0.9952	22.8 | 6.9	1.3 | 12.4	10.4 %
2-methoxy-4-vinylphenol	1.5 ppb	10 ppb	0.9952	32.3| 32.9	2.0 | 39.9	27.4 %
4-ethylguaiacol	0.75 ppb	0.75 ppb	0.9962	9.9 | 11.2	0.4 | 29.6	15.5%
Eugenol	0.75 ppb	10 ppb	0.9956	26.8 |10.2	0.9 | 22.1	15.6 %
Vanillin	1.5 ppb	7.5 ppb	0.9895	27.9| 15.4	0.8 | 20.4	14.4 %
4-ethylphenol	0.75 ppb	2 ppb	0.995	17.2 | 8.6	0.1 | 42.6	28.4 %

1.4.3. Whiskey Samples

The oak barrel flavor components, their possible origins and odors that are being targeted are summarized in Table 4 and whiskey information in Table 3.

Table 3: List of whiskeys used in this study and their alcohol content

Sample	Short name	Proof	ABV
Whiskey Blend	Blend	91.305	45.7
Jim Beam Bonded	3	100	50
Old Crow	4	86	43
Jim Beam Black Extra Aged Bourbon	6	86	43
Jim Beam Kentucky Straight Bourbon Whiskey	8	80	40
Old Grand-Dad Bonded	41	100	50
Jim Beam Red Stag	21	70	35
For Peat's Sake	228	80	40
Makers Mark	Makers Mark	90	45
Still House	Still House	69	34.5
Casa Maestri	Casa Maestri	80	40

Table 4: Possible origins and odor descriptions of the studied compounds[5–8,1]>

Compound	Origin	Odor
Geosmin	Wet grains, water, moldy barrel	Earthy, musty
2-methoxy-4-methylphenol	Oak barrel	Chocolate, clove, vanilla woody
5-methylfurfural	Oak barrel	Spicy caramel, almond, cherry
guaiacol	Oak barrel	Vanilla, almond
trans-oak lactone	Oak barrel	Spicy, coconut, vanilla, caramel
2-methoxy-4-vinylohenol	Oak barrel	Imitation vanilla, coffee, cocoa, spicy clove
4-ethylguaiacol	Oak barrel	Woody, Smokey
eugenol	Oak barrel	Powerful, warm-spicy, dry (sharp)
vanillin	Oak barrel	Vanilla, sweet, creamy
4-ethylphenol	Oak barrel	Smokey

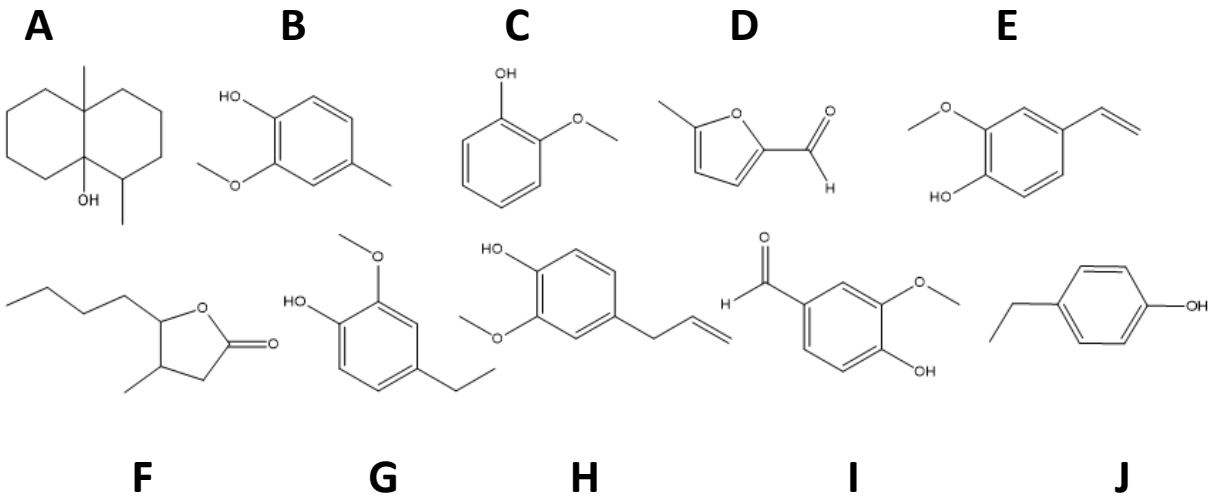


Figure 3: Structures of (A) Geosmin, (B) 2-methoxy-4-methylphenol, (C) Guaiacol, (D) 5-methylfurfurl, (E) 2-methyl-4-vinylphenol, (F) Trans Oak Lactone, (G) 4-ethylguaiacol, (H) Eugenol, (I) Vanillin and (J) 4-ethylphenol

The coupled HS-SPME-GC-MS/MS method was successful in separating the analytes of interest. Table 3 shows the quantification of each flavor component each whiskey at 40 proof. Representative TIC chromatograms are displayed in Figures 2-16 where they also state the total number of peaks in the whiskey. Each whiskey was run in triplicate.

When contamination from geosmin is present above 150 ppt, the whiskey needs to be carbon filtered to remove that taint. While the carbon filtering removes the taint, it may also remove desired flavor compounds such as the oak barrel compounds. Thus, carbon filtration is not a standard method in production for all whiskey, just those with the detected geosmin when at an undesirable level, and should be avoided to preserve the other flavor components.

A total of 11 whiskies were analyzed for 10 flavor components. Only one contained a geosmin level above the desired threshold.

Table 5a: Quantitated values of each component in 15 different whiskey samples, highest concentration for each analyte is highlighted in green

Compound / Whiskey	Geosmin (ppt)	2-methoxy-4-methylphenol	5-methylfurfural	guaiacol	trans-oak lactone
Whiskey Blend	360 ± 61	ND	110 ± 13	68 ± 0.6	210 ± 12
Jim Beam Bonded	ND	ND	150 ± 5.7	62 ± 0.5	160 ± 17
Old Crow	ND	ND	110 ± 2.1	69 ± 0.8	110 ± 25
Jim Beam Black Extra Aged B.	ND	ND	150 ± 2.1	63 ± .06	180 ± 31
Jim Beam KSBW	190 ± 84	ND	120 ± 27	60± 3.3	130± 30
Old Grand-Dad Bonded	69 ± 31	ND	120 ± 3.5	62 ± 0.6	160± 2.2
Jim Beam Red Stag	ND	220 ± 0.7	100 ± 1.1	51 ± 0.2	24 ± 3.9
For Peat's Sake	ND	550 ± 76	79 ± 1.3	240 ± 5.8	15± 0.8
Makers Mark	ND	240 ± 4.4	130 ± 2.2	63 ± 0.9	170 ± 34
Still House	ND	ND	ND	71± 1.0	33 ± 0.8
Casa Maestri	ND	150 ± 0.8	70 ± 3.4	48 ± 0.1	1.3 ± 1.2

Table 5b: Quantitated values of each component in 15 different whiskey samples, highest concentration for each analyte is highlighted in green

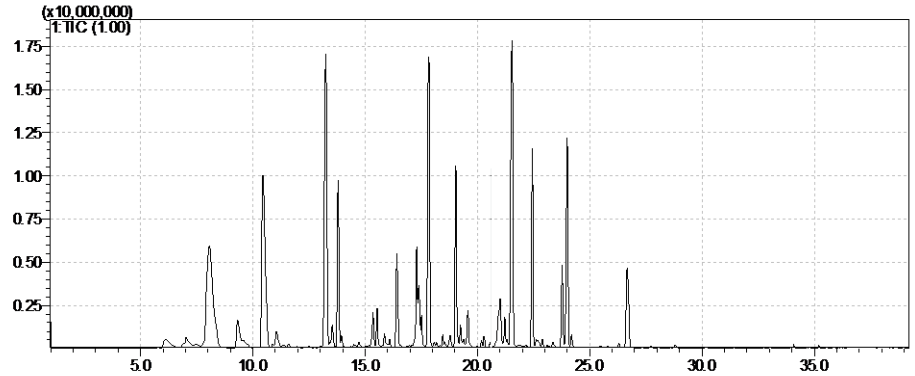
Compound / Whiskey	2-methoxy-4-vinylphenol	4-ethylguiacol	eugenol	Vanillin	4-ethylphenol
Whiskey Blend	120 ± 5.5	63 ± 2.0	440 ± 44	120 ± 2.1	58 ± 0.3
Jim Beam Bonded	140 ± 2.6	80 ± 2.3	480 ± 34	150 ± 5.2	58 ± 0.06
Old Crow	280 ± 39	170 ± 22	320 ± 48	200 ± 28	58 ± 0.03
Jim Beam Black Extra Aged B.	140 ± 12	82 ± 4.7	390 ± 51	150± 7.4	58 ± 0.05
Jim Beam KSBW	140 ± 23	82 ± 7.2	370 ± 52	140 ± 20	60 ± 3.0
Old Grand-Dad Bonded	430 ± 270	350 ± 19	4230 ± 25	330 ± 20	58 ± 0.05
Jim Beam Red Stag	77 ± 3.3	56 ± 1.5	140 ± 11	110 ± 2.8	380 ± 23
For Peat's Sake	1900 ± 53	630 ± 20	120 ± 1.6	780 ± 16	ND
Makers Mark	120 ± 14	76± 6.3	260 ± 42	130 ± 9.2	ND
Still House	210 ± 4.7	ND	6100 ± 280	150 ± 2.2	100 ± 0.9
Casa Maestri	74 ± 8.0	56 ± 3.7	80 ± 0.6	110 ± 2.2	57 ± 0.09

When conducting a comparison between the compounds of interest and all the other components in the whiskey, these oak barrel compounds consist of a very small percentage of the total composition of the whiskey in relation to their number of peaks detected. These comparisons are shown in Table 4. A representative TIC chromatogram is shown in Figure 3, additional chromatograms are in Figures S4-S14.

Table 6: Comparison of the number of peaks and their correlation to the total composition of each whiskey sample

Whiskey	# of peaks	% Everything Else	%Comps. Of Int.
Whiskey Blend	115	94	6
Jim Beam Bonded	114	94	6
Old Crow	103	95	5
Jim Beam Black Extra Aged B.	110	94	6
Jim Beam KSBW	111	96	4
Old Grand-Dad Bonded	105	92	8
Jim Beam Red Stag	97	73	27
For Peat's Sake	117	89	11
Makers Mark	116	91	9
Still House	135	93	7
Casa Maestri	100	90	10

Figure 4: Chromatogram of Jim Beam Red Stag containing 97 peaks, the lowest number of peaks



1.5 Conclusions

Consistencies in whiskey plays a vital role in distilleries producing a product that is the same every single batch. Contamination from geosmin produces a musty or moldy taste in the whiskey, even at low parts-per-trillion concentrations. This contamination can come from water, moist grains, or the barrel used in aging. Quantifying the geosmin contamination is very important in ensuring a consistent and enjoyable beverage in the event that carbon filtration is necessary prior to bottling for a fault-free product.

On the other hand, quantification of oak barrel compounds can provide insight into the more pronounced flavors that come through in the whiskey. By quantifying and identifying these compounds, a better characterization can be produced for better advertisement of the beverage. The quantity of each compound can vary based on the barrel used, if it is new or used, the origin of the wood and the exact placement and environment of each barrel when a beverage is aging. Quality control of oak volatiles, in this regard, is an important part of whiskey production, to help ensure consistent flavor in a product.

A HS-SPME-GC-MS method was developed for the simultaneous quantification of geosmin, 2-methoxy-4-methylphenol, 5-methylfurfural, guaiacol, trans-whiskey lactone, 2-methoxy-4-vinylphenol, 4-ethylguaiacol, eugenol, vanillin, and 4-ethylphenol. This method was developed to point towards a possible quality control process. Geosmin has a threshold in which carbon filtration must be used, but the other compounds are meant to provide a better understanding of the transference of oak flavors from the barrel to the whiskey. This method could be further developed to identify and quantify other compounds that present themselves at any point during the production of the whiskey product.

1.6 Supporting Information

Figure S5: Chromatogram of a whiskey blend containing 116 peaks

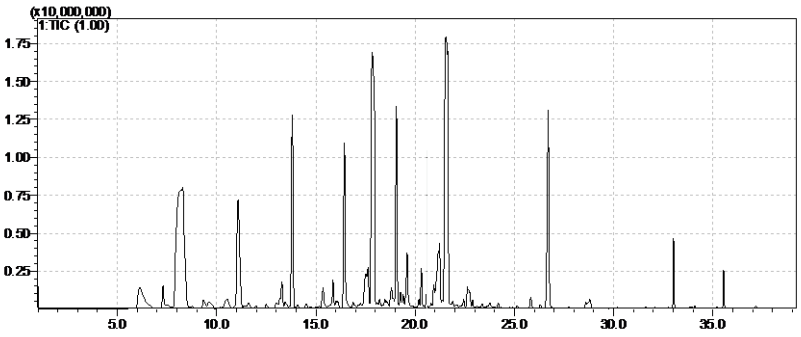


Figure S6: Chromatogram of For Peat's Sake containing 117 peaks

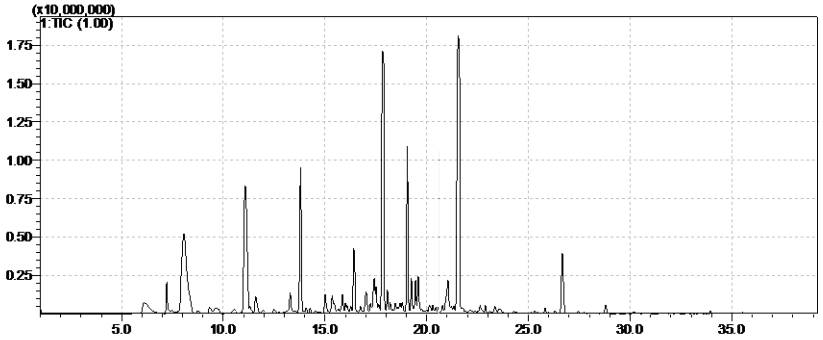


Figure S7: Chromatogram of Jim Beam Kentucky Straight Bourbon Whiskey containing 111 peaks

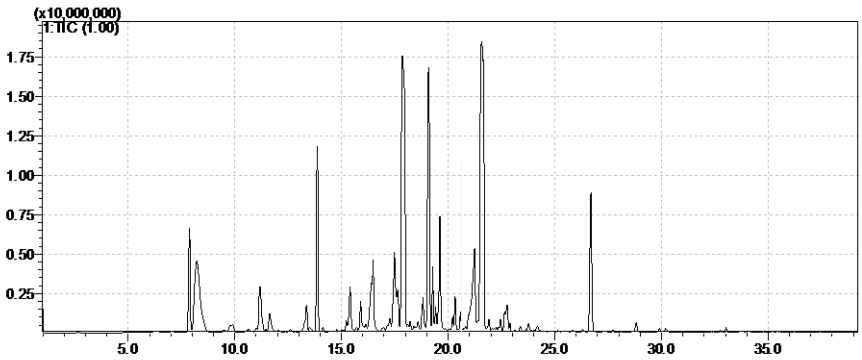


Figure S8: Chromatogram of Jim Beam Black Extra Aged Bourbon containing 110 peaks

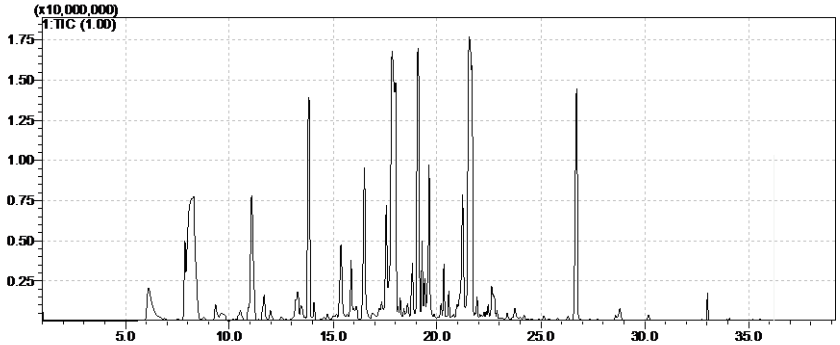


Figure S9: Chromatogram of Jim Beam Red Stag containing 97 peaks, the lowest number of peaks

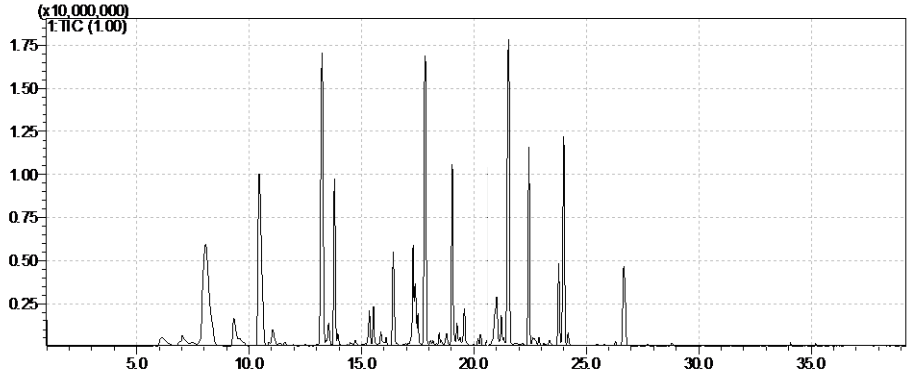


Figure S10: Chromatogram of Old Grand-Dad Bonded containing 105 peaks

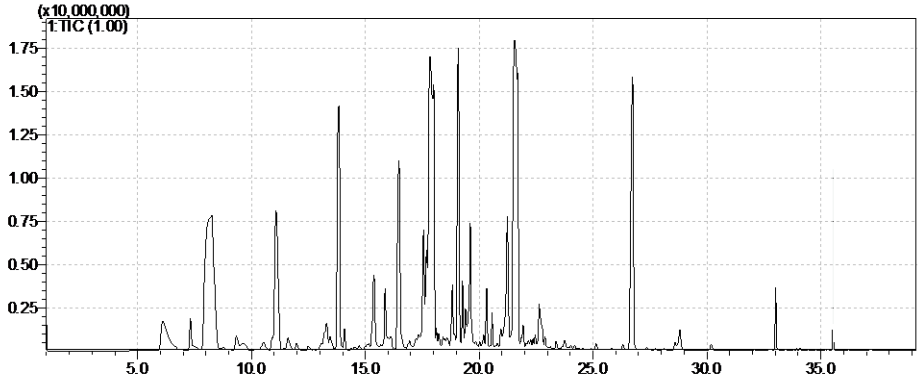


Figure S11: Chromatogram of Maker's Mark containing 116 peaks

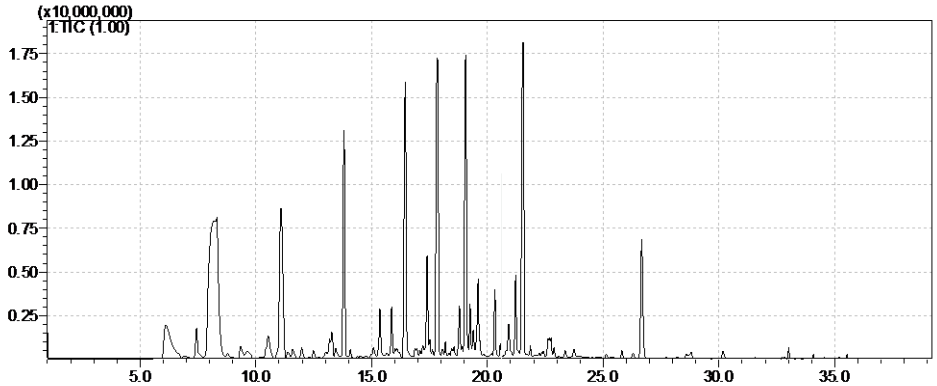


Figure S12: Chromatogram of Stillhouse containing 135 peaks, the largest number of peaks

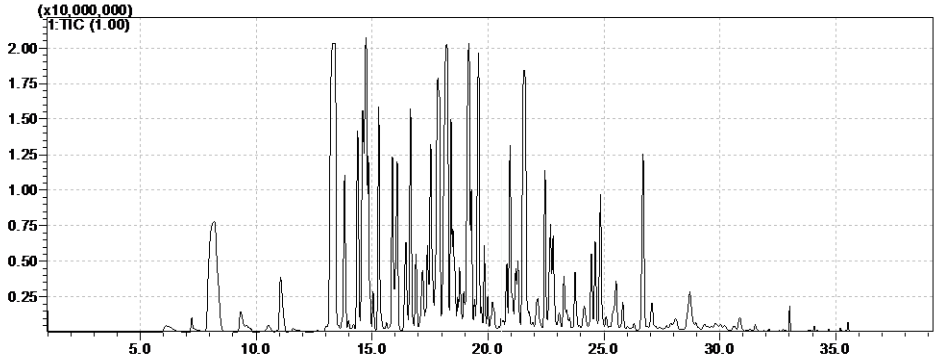


Figure S13: Chromatogram of Casa Maestri containing 100 peaks

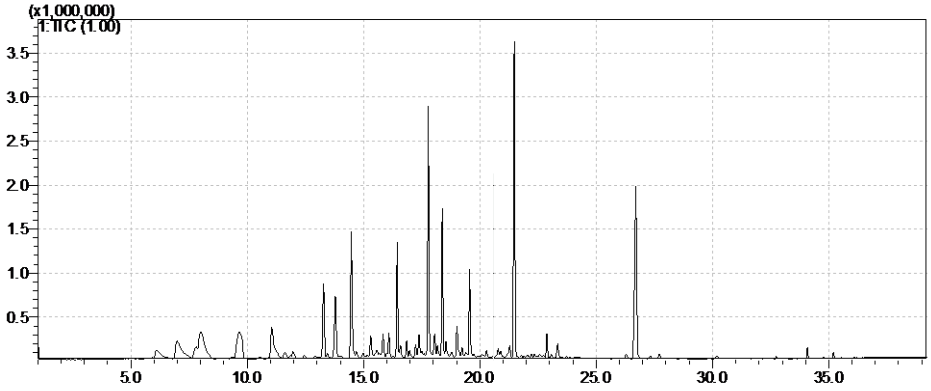


Figure S14: Chromatogram of Old Crow containing 103 peaks

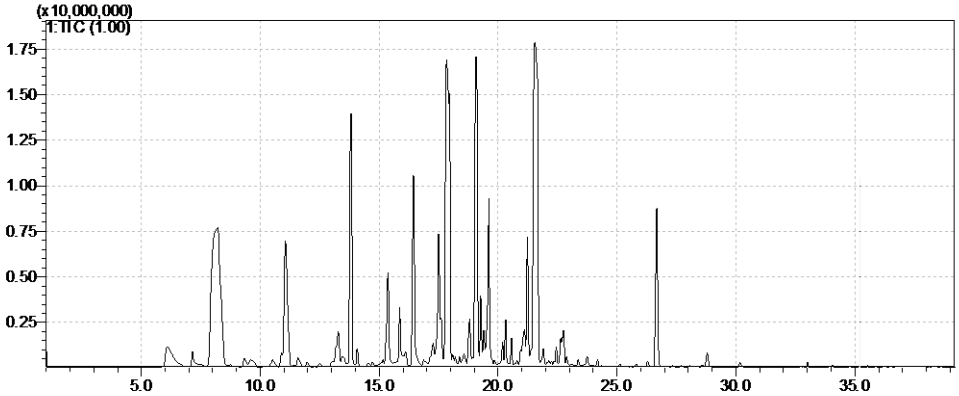
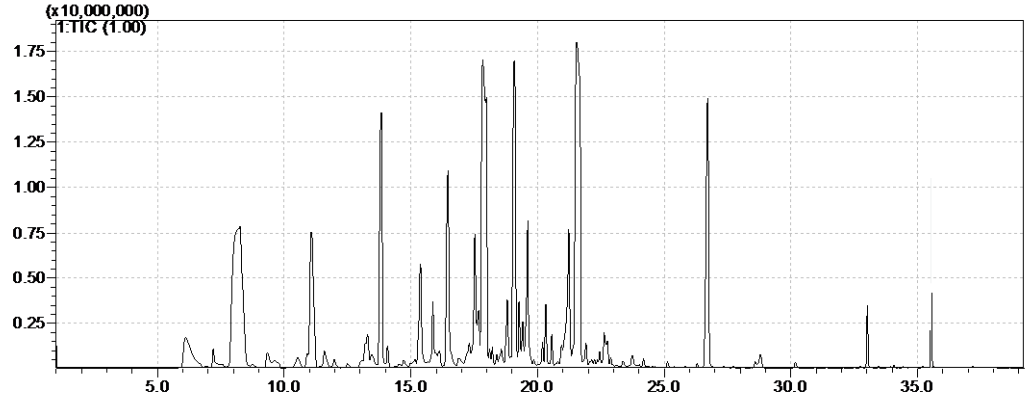
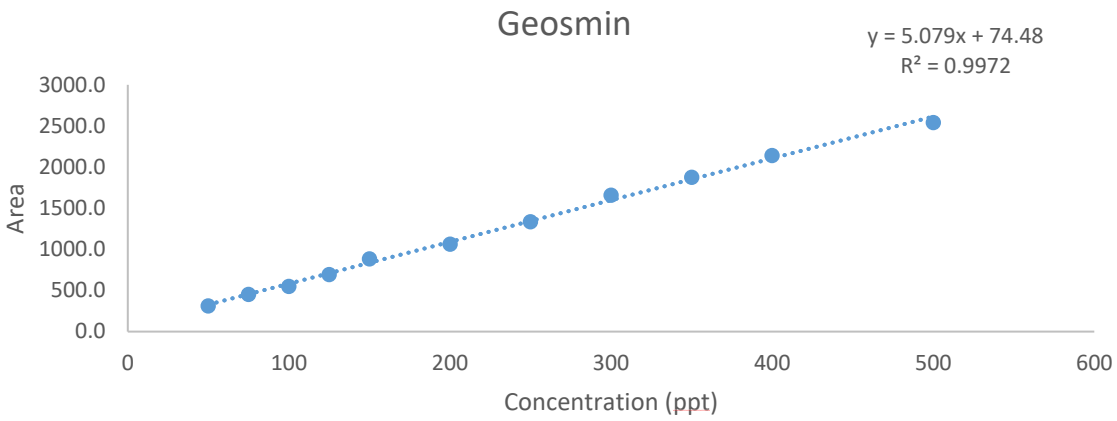
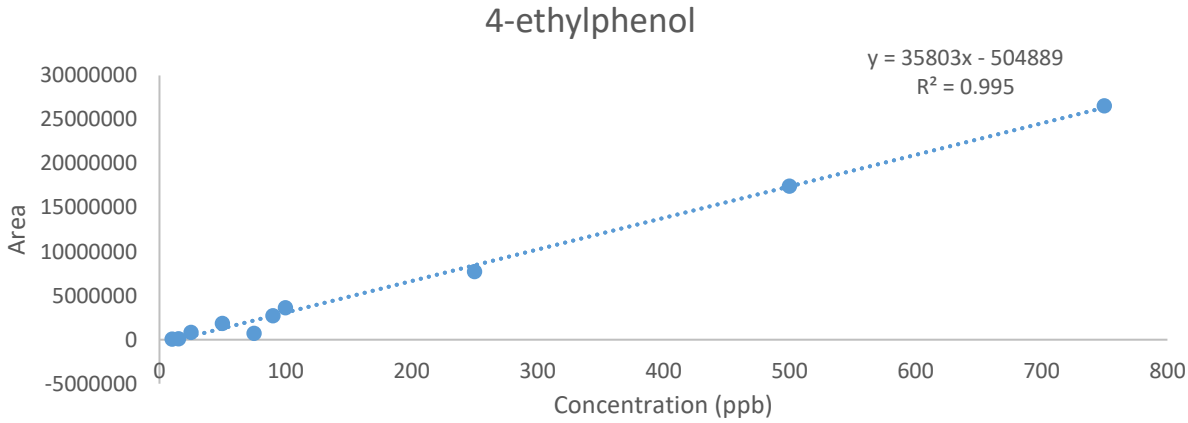
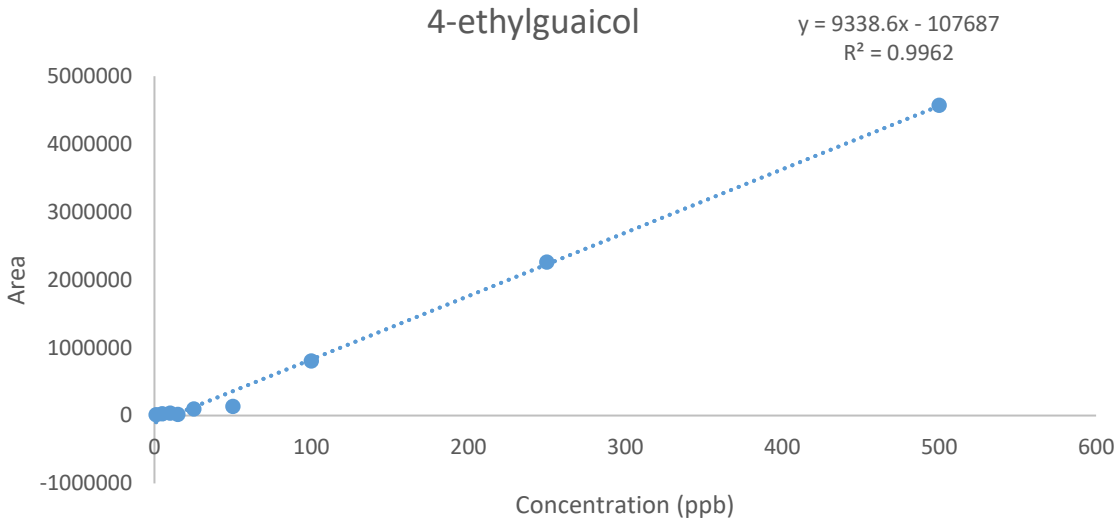


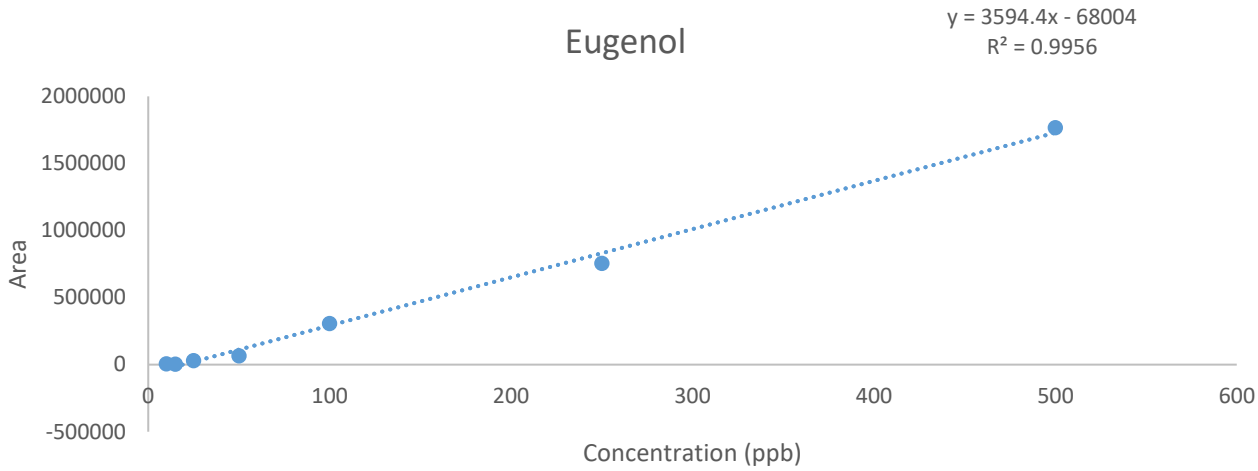
Figure S15: Chromatogram of Jim Beam Bonded containing 114 peaks

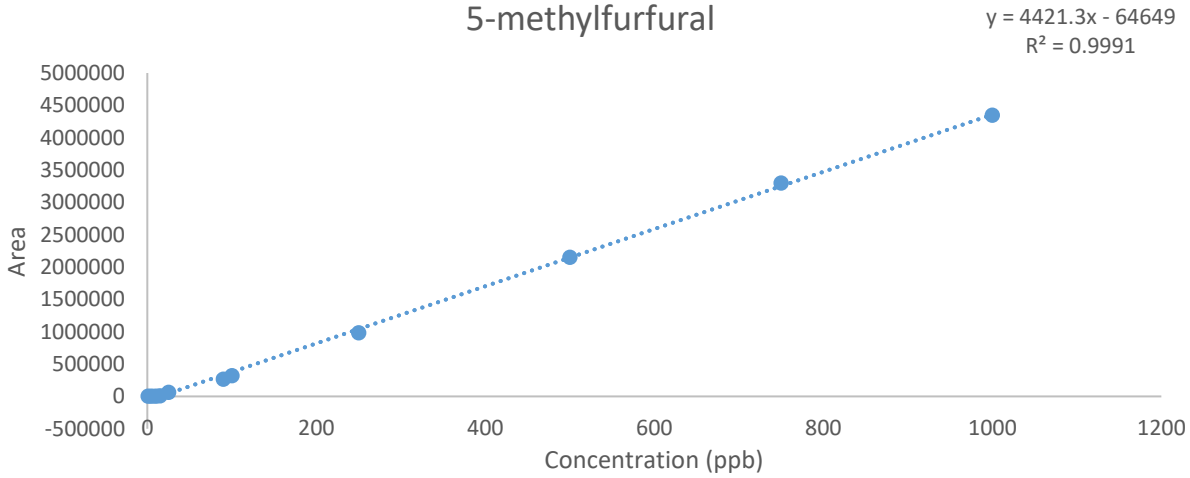


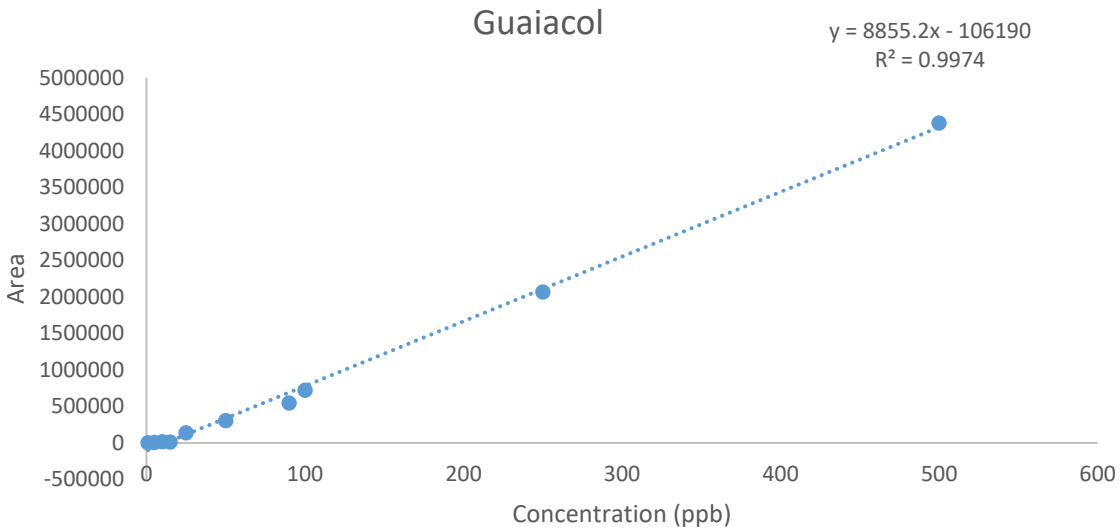

Geosmin
y = 5.079x + 74.48
R² = 0.9972
Area
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
0
100
200
300
400
500
600
Concentration (ppt)

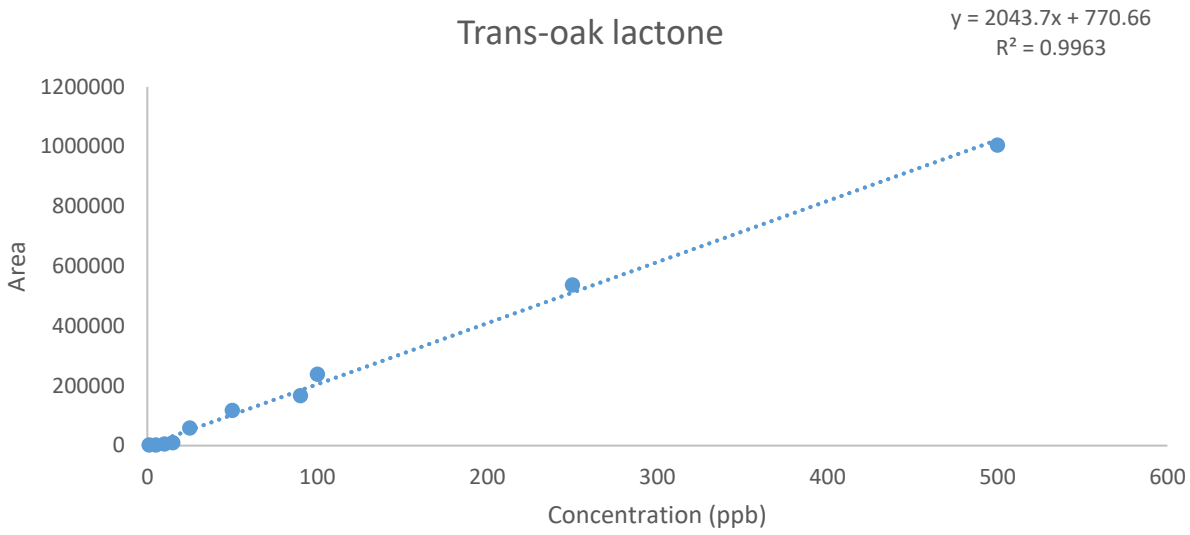

4-ethylphenol
y = 35803x - 504889
R² = 0.995
Area
30000000
25000000
20000000
15000000
10000000
5000000
0
-5000000
0
100
200
300
400
500
600
700
800
Concentration (ppb)

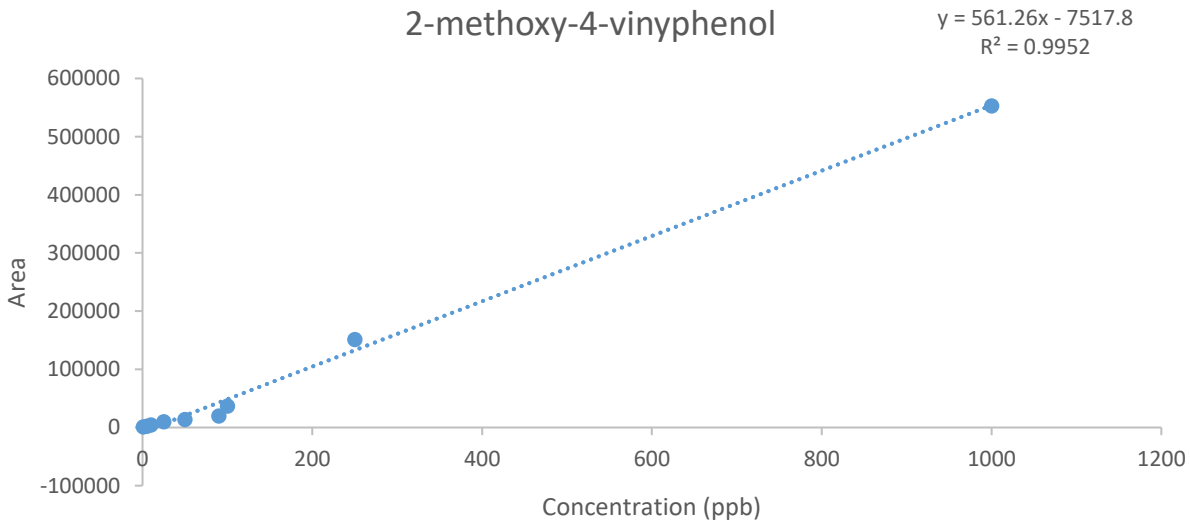

4-ethylguaicol
y = 9338.6x - 107687
R² = 0.9962
Area
5000000
4000000
3000000
2000000
1000000
0
-1000000
0
100
200
300
400
500
600
Concentration (ppb)

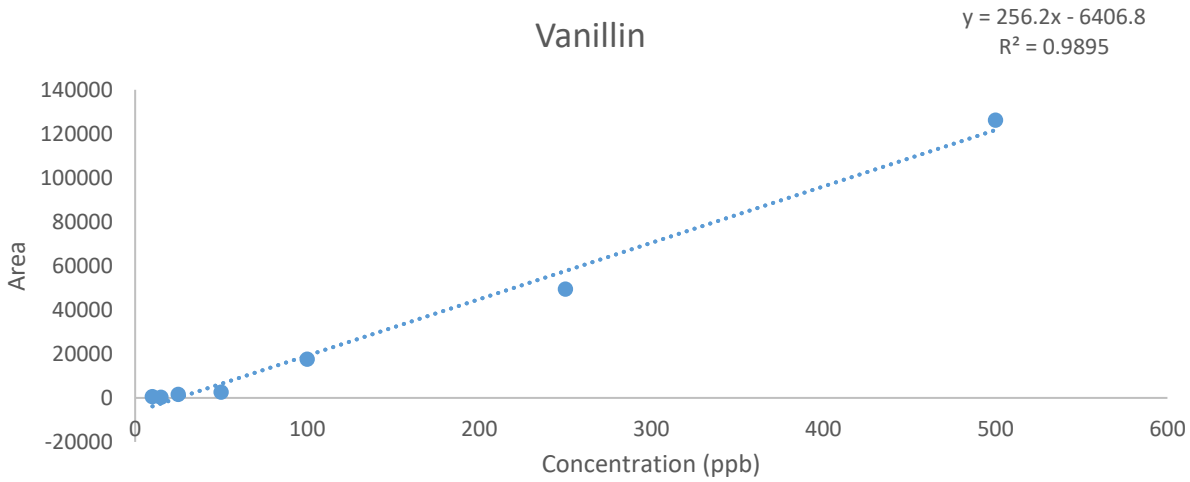

Eugenol
y = 3594.4x - 68004
R² = 0.9956
Area
Concentration (ppb)
2000000
1500000
1000000
500000
0
-500000
0
100
200
300
400
500
600


5-methylfurfural
y = 4421.3x - 64649
R² = 0.9991
Area
Concentration (ppb)
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
0
200
400
600
800
1000
1200


Guaiacol
y = 8855.2x - 106190
R² = 0.9974
Area
Concentration (ppb)
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
0
100
200
300
400
500
600


Trans-oak lactone
y = 2043.7x + 770.66
R² = 0.9963
Area
Concentration (ppb)
0
200000
400000
600000
800000
1000000
1200000
0
100
200
300
400
500
600


2-methoxy-4-vinyphenol
y = 561.26x - 7517.8
R² = 0.9952
Area
Concentration (ppb)
-100000
0
100000
200000
300000
400000
500000
600000
0
200
400
600
800
1000
1200


Vanillin
y = 256.2x - 6406.8
R² = 0.9895
Area
Concentration (ppb)
-20000
0
20000
40000
60000
80000
100000
120000
140000
0
100
200
300
400
500
600

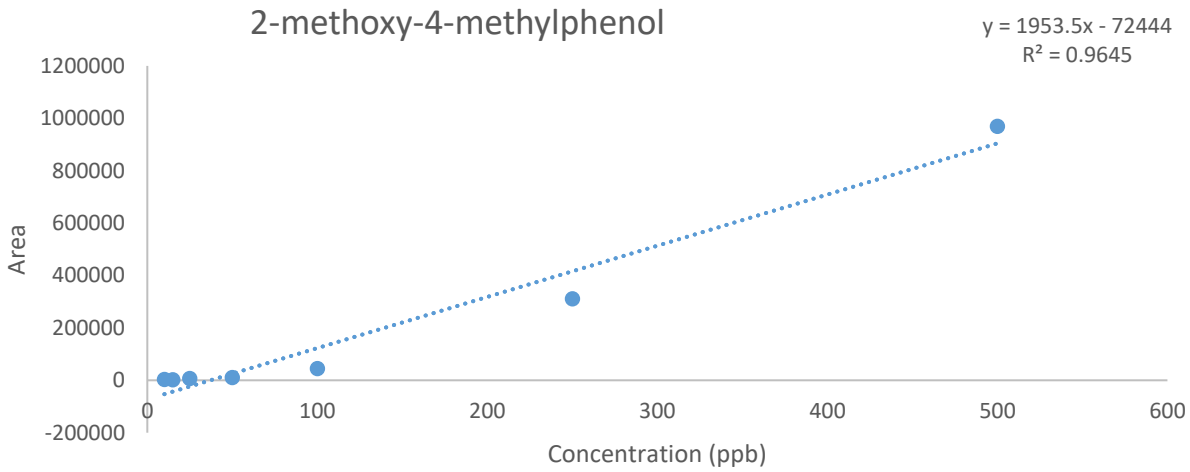


Figure S16: Calibration Curves for Geosmin, 2-methoxy-4-methylphenol, Guaiacol, 5-methylfurfurl, 2-methyl-4-vinylphenol, Trans Oak Lactone, 4-ethylguaiacol, Eugenol, Vanillin and 4-ethylphenol

Table 7-1S: Concentration levels (ng/L (geosmin) and mg/L for other compounds) applied to each calibration curve for the studied fault compounds.

stock used	final conc (ppb)	Point	2m4mp	gua	2m4vp	4eg	eu	isoeu	oak	vanillin	4ep	5mf	geo	5% EtOH	water
1 ppm	1 ppb	1	5	5	5	5	5	5	5	5	5	5	0	250	4700
1 ppm	5 ppb (50 ppt)	2	25	25	25	25	25	25	25	25	25	25	250	250	4250
1 ppm	10 ppb (75 ppt)	3	50	50	50	50	50	50	50	50	50	50	375	250	3875
1 ppm	15 ppb (100 ppt)	L	75	75	75	75	75	75	75	75	75	75	500	250	3500
1 ppm	25 ppb (125 ppt)	4	125	125	125	125	125	125	125	125	125	125	6.25	250	3493.75
1 ppm	50 ppb (150 ppt)	5	250	250	250	250	250	250	250	250	250	250	7.5	250	2242.5
1 ppm	60 ppb (200 ppt)	M	300	300	300	300	300	300	300	300	300	300	10	250	1740
1 ppm	75 ppb (250 ppt)	6	375	375	375	375	375	375	375	375	375	375	12.5	250	987.5
1 ppm	90 ppb (300 ppt)	7	450	450	450	450	450	450	450	450	450	450	15	250	235
100 ppm	100 ppb (350 ppt)	H	5	5	5	5	5	5	5	5	5	5	17.5	250	4682.5
100 ppm	250 ppb (400 ppt)	8	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	20	250	4605
100 ppm	500 ppb (500 ppt)	9	25	25	25	25	25	25	25	25	25	25	25	250	4475

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Biographical Information

Hailey Jarzynka received her Bachelor of Science degree in Chemistry from Stephen F. State University in 2020. While there, she worked as an undergraduate researcher for three years under Dr. Michelle Harris' Biochemistry lab. She studied the biotransformation reactions of pro-chiral ketones and observing their antimicrobial properties. Hailey began her graduate school journey in fall of 2020 at the University of Texas at Arlington. She joined Dr. Kevin Schug's Analytical Chemistry lab in the summer of 2021 and began by aiding Dr. Haile Ratcliffe in her study of fault compounds in whiskey. Hailey's research expanded on Dr. Ratcliffe's initial fault compound study. This study was expanded to contain oak barrel compounds and quantify them through headspace –solid phase micro extraction – gas chromatography – mass spectrometry. She collaborated with a local brewery for whiskey samples. She will graduate with her Masters of Science in Chemistry from the University of Texas at Arlington in December of 2021 and will start a career as a Forensic Chemist.