GCmate II

Analysis of Scotch Whiskey and Tequila Samples by Solid-Phase Microextraction and High-Resolution GC/MS

Summary: Solid-phase microextraction (SPME) is a convenient sample preparation method for extracting organic compounds from aqueous samples. The combination of SPME with gas chromatography and high-resolution mass spectrometry provides powerful capabilities for the analysis of alcoholic beverages.

Two samples of Scotch whiskey and one tequila sample were sampled by using solid-phase microextraction for analysis by high-resolution GC/MS. Sample 1 was a blended 12-year old light Scotch whiskey, while sample 2 was a 12-year old single-malt light Scotch whiskey. The tequila sample was a popular brand that is widely sold in the USA. Compounds were extracted and identified by GC/MS with library search. Exact mass measurements provided elemental compositions for molecular ions and fragment ions.

Solid-Phase Microextraction: A Supelco 2cm-50/30µm DVB Carboxen/PDMS StableFlex SPME Fiber Assembly mounted in a manual SPME holder was immersed in each sample for approximately 5 minutes and then desorbed into the GC injector port at 250°C for 2 minutes.

Gas Chromatography: An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2 minutes, then ramp at 20°C per minute to 260°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrometry: A JEOL *GCmate II* benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with *TSS-2000*[1] software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 500 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from *m/z* 65 to *m/z* 350 at 1 second per scan. Perfluorokerosene was continuously introduced as an internal reference compound. Chemical ionization mass spectra were acquired by using ammonia reagent gas to selectively ionize polar compounds (ketones, acids, esters). The component detection algorithm (CODA[2]) was used to reduce chemical background and locate small peaks. Compounds were identified by searching the

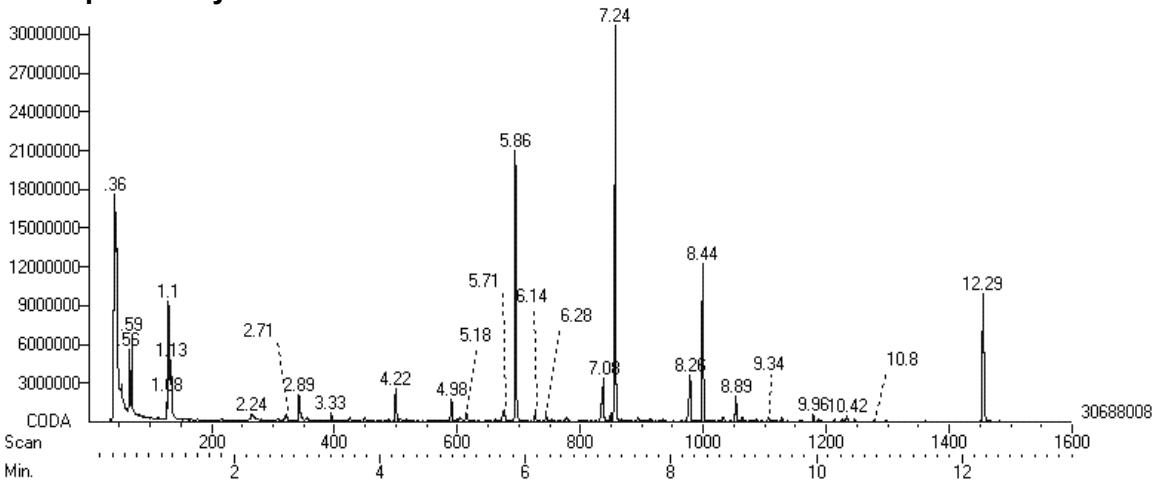
NIST 2000 mass spectral database. Elemental compositions were calculated from the exact mass measurements.

Results: Over 50 compounds were identified in the whiskey samples and many additional compounds were identified in tequila. A list of compounds and retention times is shown in Table I. Exact mass measurements provided elemental compositions that were generally within 0.002μ for molecular ions and/or fragment ions for each compound. Exact mass measurements provided confirmation for a compound's expected elemental composition and made it easy to assign structures to fragment ions in the EI mass spectra.

Table I. Components Identified in Scotch Whiskey (many common to tequila sample)

Compound	R.T. (min)	Comment
Ethanol	0.36	
1-Propanol	0.45	
Ethyl acetate	0.56	
1-propanol, 2-methyl	0.59	
Methane, diethoxy	0.76	
Propanoic acid, ethyl ester	0.95	
Ethane, 1,1-diethoxy	1.08	
1-Butanol, 3-methyl	1.1	
1-Butanol, 2-methyl	1.12	
Propanoic acid, 2-methyl, ethyl ester	1.32	
Acetic acid, 2-methylpropyl ester	1.49	
Butanoic acid, ethyl ester	1.83	
Furfural	2.24	
Butanoic acid, 2-methyl, ethyl ester	2.54	
Butanoic acid, 3-methyl, ethyl ester	2.58	
Propane,1,1-diethoxy, 2-methyl	2.68	
Butane, 1,1-diethoxy	2.68	
p-Xylene	2.71	From plastic sampling tube?
1-Butanol, 3-methyl acetate	2.89	
1-Butanol, 2-methyl acetate	2.93	
Butane, 1,1-diethoxy-3-methyl	3.78	
Pentane,1-(1-ethoxyethoxy)	3.98	
Hexanoic acid, ethyl ester	4.22	
Cyclotrisiloxane, octamethyl	4.98	Contamination from GC column or septum
Hexane, diethoxy	5.06	
Heptanoic acid, ethyl ester	5.08	
Phenylethyl alcohol	5.18	
Octanoic acid	5.74	
Octanoic acid, ethyl ester	5.86	
Cyclotetrisiloxane, octamethyl	6.14	Contamination from GC column or septum
Hexanoic acid,1,1-dimethylpropyl ester	6.24	
Acetic acid, 2-phenylethyl ester	6.28	
Heptanol, 3-methyl-1-heptanol	6.4	
Nonanoic acid, ethyl ester	6.57	
cis-3-methyl-4-octanolide	6.75	
n-Caprylic acid, isobutyl ester	6.92	
n-Decanoic acid	7.11	
Ethyl-9-decenoate	7.18	
Decanoic acid, ethyl ester	7.24	
Octanoic acid, 3-methylbutyl ester	7.57	
Formic acid, decyl ester	7.71	
n-Capric acid, isobutyl ester	8.15	
Dodecanoic acid	8.27	
Dodecanoic acid, ethyl ester	8.44	
Pentadecanoic acid, 3-methyl, butyl ester	8.72	
Tetradecanoic acid	9.34	
Tetradecanoic acid, ethyl ester	9.52	
Hexadecanol	9.96	
Z-10-tetradecan-1-ol acetate	10.25	
n-Hexadecanoic acid	10.35	
Hexadecenoic acid, ethyl ester	10.41	
Hexadecanoic acid, ethyl ester	10.51	
Nonanoic acid(?), phenylethyl ester	10.8	Chain length uncertain, no molecular ion in EI
Bis(2-ethylhexyl) adipate	12.29	Plasticizer, from packaging?

Sample 1: 12-year old blended



Sample 2: 12-year old single-malt

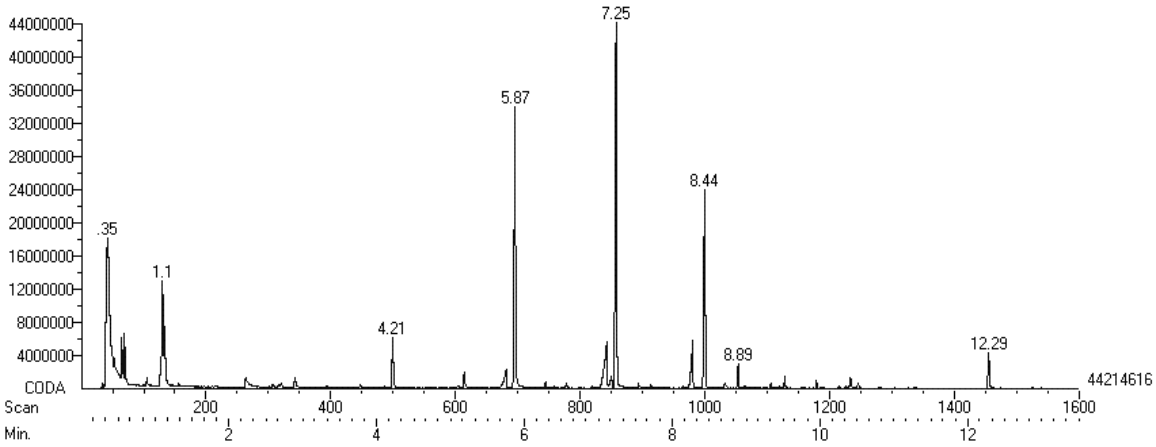
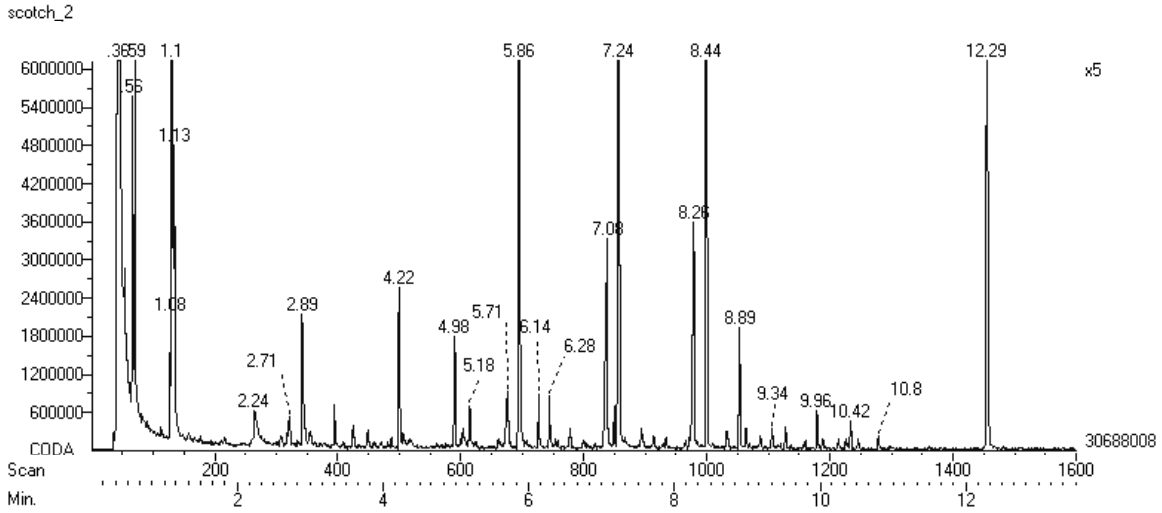


Figure 1. Total-ion current chromatograms for the Scotch samples.

The two samples were similar in color, odor, and taste. Small differences were observed in the relative ratios of certain components. For example, sample 1 had a higher level of 3-methyl, 1-butanol while sample 2 had higher levels of ethyl dodecanoate, phenylethyl alcohol and tetradecanoic acid.

Sample 1



Sample 2

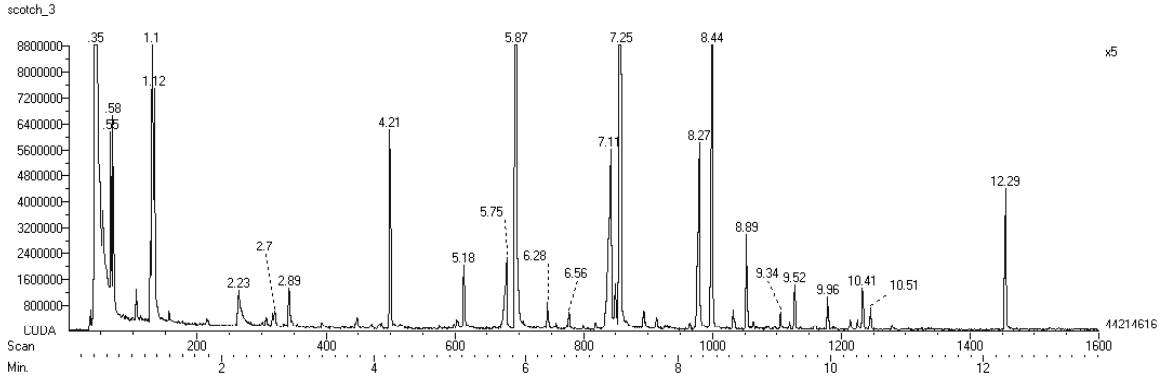


Figure 2. Enlarged view of the chromatograms in Figure 1 to show low-level components.

Measured mass spectrum of component eluting at 6.28 minutes.

Mass spectrum from library database.

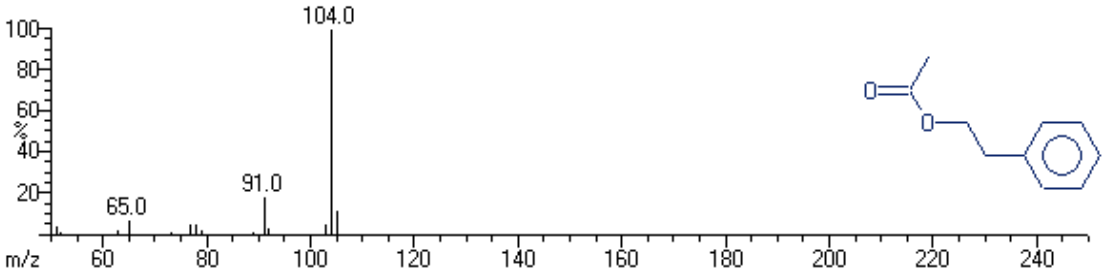
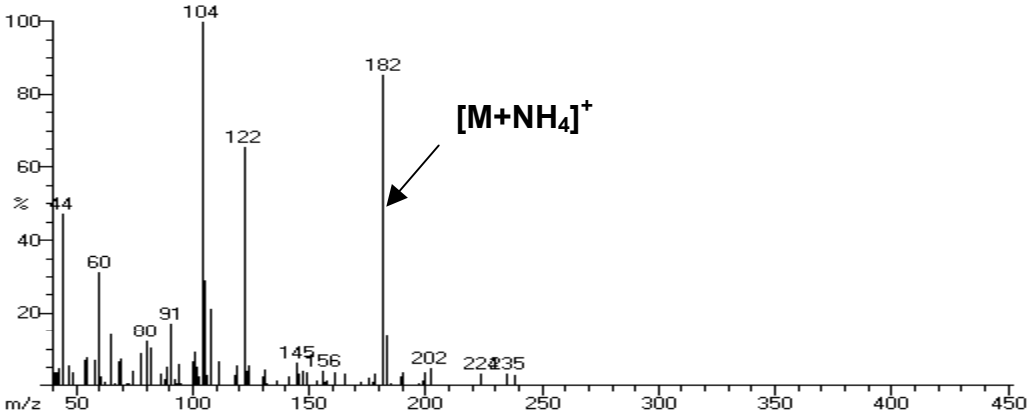


Figure 3. Mass spectrum and library search result for component at 6.28 minutes.

Ammonia CI mass spectrum of component at retention time 6.28 min.



Ammonia CI confirmed the molecular weight for many polar compounds that did not produce a molecular ion in EI mode.

Figure 4. Ammonia CI mass spectrum of the compound in Figure 3 confirms the molecular weight (164μ) by the presence of the [M+NH4]+ peak. Note that the molecular ion is not observed in the EI mass spectrum of this compound.

Table 2: Typical High resolution Exact Mass Measurements and Elemental Composition for Sample 1.

Compound	Meas. m/z	Composition	Ion	Calc. m/z	Error (mmu)
Ethyl acetate	88.05077	C4 H8 O2	M+.	88.05243	-1.7
1-Propanol, 2-methyl	74.07131	C4 H10 O	M+.	74.07317	-1.9
Diethoxyethane	103.0752	C5 H11 O2	[M-CH3]+	103.0759	-0.7
Furfural	96.02313	C5 H4 O2	M+.	96.02113	2
Furfural	95.01513	C5 H3 O2	[M-H]+	95.01331	1.8
1-Butanol, 3-methyl acetate	87.04336	C4 H7 O2	Fragment	70.07825	-0.2
1-Butanol, 3-methyl acetate	70.07805	C5 H10	Fragment	87.04461	-1.2
Hexanoic acid, ethyl ester	88.05026	C4H8O2	Fragment	88.05243	-2.2
Hexanoic acid, ethyl ester	99.07999	C6 H11 O	Fragment	99.081	-1
Hexanoic acid, ethyl ester	101.0588	C5 H9 O2	Fragment	101.0603	-1.5
Hexanoic acid, ethyl ester	115.0717	C6 H11 O2	Fragment	115.0759	-4.2
Hexanoic acid, ethyl ester	117.0923	C6 H13 O2	Fragment	117.0916	0.7
Hexanoic acid, ethyl ester	144.1134	C8 H16 O2	M+.	114.115	-1.6
Phenylethyl alcohol	122.0713	C8 H10 O	M+.	122.0732	-1.9
Phenylethyl alcohol	92.06038	C7 H8	Fragment	92.0626	-1
Phenylethyl alcohol	91.05377	C7 H7	Fragment	91.05478	-1
Decanoic acid	172.1481	C10 H20 O2	M+.	172.1463	1.8
Decanoic acid, ethyl ester	200.1776	C12 H24 O2	M+.	200.1776	0
Dodecanoic acid	200.1764	C12 H24 O2	M+.	200.1776	-1.2
Dodecanoic acid, ethyl ester	228.2078	C14 H28 O2	M+.	228.2089	-1.1

Exact mass measurements allow confirmation of elemental compositions for analytes and fragment ions.

Table 3: Exact mass measurements used to automatically assign fragment compositions and probable structures for ethyl octanoate.

Fragment Series Analysis — Error limits: 10 ppm (Listed as mmu); 5 mmu if < m/z 500; 20 mmu if > m/z 2000

Series formula: Cn H2n-1 **Description: Alkenes/ cycloalkanes**

	Peak	Target	Diff.	Abundance
5	69.0694	69.07042	1.022	2.739367
6	83.0856	83.08607	0.465	3.202421
7	97.1032	97.10172	-1.488	1.831384

Series formula: Cn H2n-1 CO **Description: Alkenyl-/ cycloalkyl C=0(specific); diunsat./cyc. alc./ ethers**

	Peak	Target	Diff.	Abundance
4	83.0494	83.04969	0.29	3.021625
5	97.0677	97.06534	-2.365	2.812579
7	125.0974	125.0966	-0.763	2.735364

Series formula: Cn H2n-1 O2 **Description: Acids/ esters/ cyclic acetals/ ketals (some specific)**

	Peak	Target	Diff.	Abundance
3	73.027	73.02895	1.953	12.66455
4	87.0432	87.0446	1.404	6.874841
5	101.0593	101.0602	0.946	50.58382
6	115.0712	115.0759	4.7	11.74692
7	129.0905	129.0916	1.053	12.77449
9	157.121	157.1229	1.862	1.392577

Series formula: O **Description: 2**

	Peak	Target	Diff.	Abundance
	70.0763	70.07825	1.945	1.411645
	84.0914	84.09389	2.495	1.166582

Series formula: H2n-2 (OE) **Description: Cycloalkyl=|=HY/cycloalkyl=|=YY'/CnH2n-2=|=YY'/ket.lossCnH2n+H20**

	Peak	Target	Diff.	Abundance
	70.0763	70.07825	1.945	1.411645
	84.0914	84.09389	2.495	1.166582

Series formula: Cn H2n (OE) **Description: CHR=|=CR'CHR"~Z~H/ cycloalkanes/ CnH2n=|=RY/ H~CnH2n~Y***

	Peak	Target	Diff.	Abundance
5	70.0763	70.07825	1.945	1.411645
6	84.0914	84.09389	2.495	1.166582

Series formula: Cn H2n-2 O (OE) **Description: 2-Alkylcycloalkanone/cyclobutanones(spec)/98fromY(CH2)n>5-CO-Y**

	Peak	Target	Diff.	Abundance
4	70.0407	70.04186	1.16	10.73583
5	84.0585	84.05751	-0.992	2.92746
6	98.0732	98.07316	-0.038	3.432183

Series formula: Cn H2n O2 (OE) **Description: ROCOCHR'~Z~H/ H~Z~OCOCHR~Z'~H/ CnH2nO2=|=RY**

	Peak	Target	Diff.	Abundance
3	74.0334	74.03678	3.38	2.607301
4	88.0505	88.05243	1.93	100
7	130.0983	130.0994	1.083	2.121411
8	144.1185	144.115	-3.464	1.351145(13C?)
10	172.1432	172.1463	3.143	5.967094

Neutral losses from m/z 172.1432

Loss formula: RCnH2n-1

RCOOR'-spec. rear. loss and also carbonates and amides and lrg. ketones and etc.

Loss	Peak	Target	Diff.	Abund.
83.08607	89.0577	89.05714	-0.565	7.272215 13C?

Loss formula: CnH2n

RCH2COCH2R-spec. rearr. loss and many unsat. grps. and retro-Diels-Alder

Loss	Peak	Target	Diff.	Abund.
42.04695	130.0983	130.0963	-2.045	2.121411
70.07825	102.0613	102.065	3.654	4.01977 13C?
84.09389	88.0505	88.04931	-1.19	100
98.10954	74.0334	74.03366	0.259	2.607301

Loss formula: CnH2n+1

Alkyl loss (a-cleave or branched site favored) and cycloalkyl elim w/ H rearr.

Loss	Peak	Target	Diff.	Abund.
15.02347	157.121	157.1197	-1.266	1.392577
43.05476	129.0905	129.0884	-2.06	12.77449
57.07042	115.0712	115.0728	1.587	11.74692
71.08607	101.0593	101.0571	-2.167	50.58382
85.10172	87.0432	87.04149	-1.709	6.874841
99.11736	73.027	73.02584	-1.16	12.66455

Loss formula: OH

Acids and oximes and phenols; rearrangement (e.g. o-NO2C6H4CH3)

Loss	Peak	Target	Diff.	Abund.
45.03404	127.1111	127.1092	-1.938	56.35329

Loss formula: CnH2n+1O

R-|-OR'; RC-|-OR'

Loss	Peak	Target	Diff.	Abund.
45.03404	127.1111	127.1092	-1.938	56.35329

Loss formula: CnH2n+1 CO2

R-|-OCOR'; R-|-COOR' (stable R+ and small R' only)

Loss	Peak	Target	Diff.	Abund.
87.0446	85.0995	85.0986	-0.9	1.243797

Loss formula: H2O

Alcohols (primary favored); higher MW aldehydes and ketones and ethers

Loss	Peak	Target	Diff.	Abund.
74.07316	98.0732	98.07005	-3.151	3.432183
88.08881	84.0585	84.0544	-4.105	2.92746
102.1045	70.0407	70.03875	-1.953	10.73583

Loss formula: CnH2n+1 OH

Loss ROH frm R'CH2OR and frm R'COOR w/ labile H; these w/ further loss CnH2n (n>

Loss	Peak	Target	Diff.	Abund.
74.07316	98.0732	98.07005	-3.151	3.432183
88.08881	84.0585	84.0544	-4.105	2.92746
102.1045	70.0407	70.03875	-1.953	10.73583

Loss formula: CO+CnH2n+1 OH

Loss of CO + HOR from R'COOR w/ labile H; cyclic -OCHRO-

Loss	Peak	Target	Diff.	Abund.
88.05242	84.0914	84.09078	-0.618	1.166582
102.0681	70.0763	70.07513	-1.167	1.411645

Loss formula: CnH2n+1 COOH

R'COO-|-R-H (stable R+. and small R')

Loss	Peak	Target	Diff.	Abund.
88.05243	84.0914	84.09077	-0.626	1.166582
102.0681	70.0763	70.07513	-1.175	1.411645

Loss formula: CH3+H2O

Some alcohols

Loss	Peak	Target	Diff.	Abund.
47.04969	125.0974	125.0935	-3.883	2.735364
61.06534	111.0729	111.0779	4.967	1.136449
89.09663	83.0494	83.04657	-2.831	3.021625

Tequila sample – CODA Chromatogram

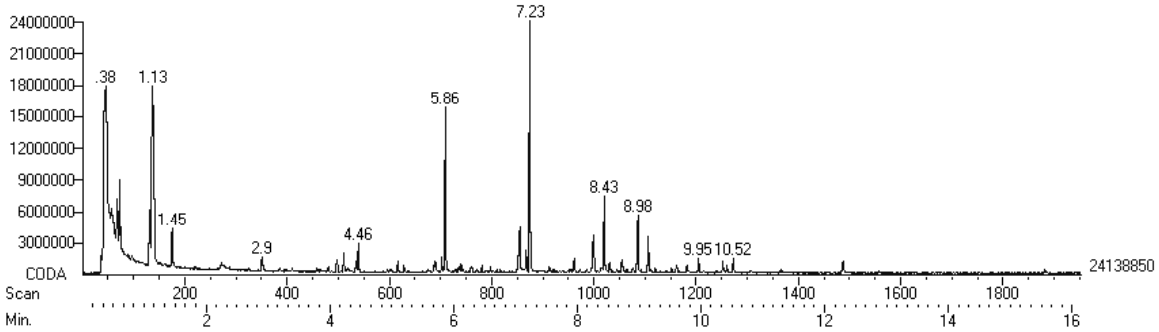


Figure 5. Component detection algorithm (CODA) chromatogram for tequila sample.

Table 4: Additional Compounds found in tequila (see also Scotch whiskey table)

Compound	RT	MW	Composition
1-Butanol	0.74	74	C4 H10 O
S-(+)-Propylene glycol	1.47	76	C3 H8 O2
2,2-Pentadienoic acid, ethyl ester	3.18	126	C7H10 O2
2-Carene	4.12	136	C10 H16
p-Cymene	4.42	134	C10 H14
D-limonene	4.46	136	C10 H16
Linalool	5.1	154	C10 H18 O
Terpineol	5.8	154	C10 H18 O
R-(+)-a-Citronellol	6.09	156	C10 H20 O
3-BHA	6.12	180	C11 H16 O2
a-Phenylethylbutyrate	6.29	192	C12 H16 O2
(+)-Cycloisosativene	7.07	204	C15 H24
a-Farnesene	7.61	204	C15 H24
Phenol, di-2,5-t-butyl	7.95	206	C14 H22 O
Nerolidol	8.27	222	C15 H26 O
Cubenol	8.52	222	C15 H26 O
Nerolidol isomer	8.65	222	C15 H26 O
2,3-dihydrofarnesol	8.97	224	C15 H28 O
trans-Farnesol	9.15	222	C15 H26 O
trans,trans-Farnesol	9.26	222	C15 H26 O
1,5,9-decatriene,2,3,5,8-tetramethyl	9.61	192	C14 H24
All-trans-farnesyl acetate	9.77	264	C17 H28 O2
Farnesyl acetate	9.77	264	C17 H28 O2

TIC, CODA, and mass Chromatogram for Terpene m/z 93

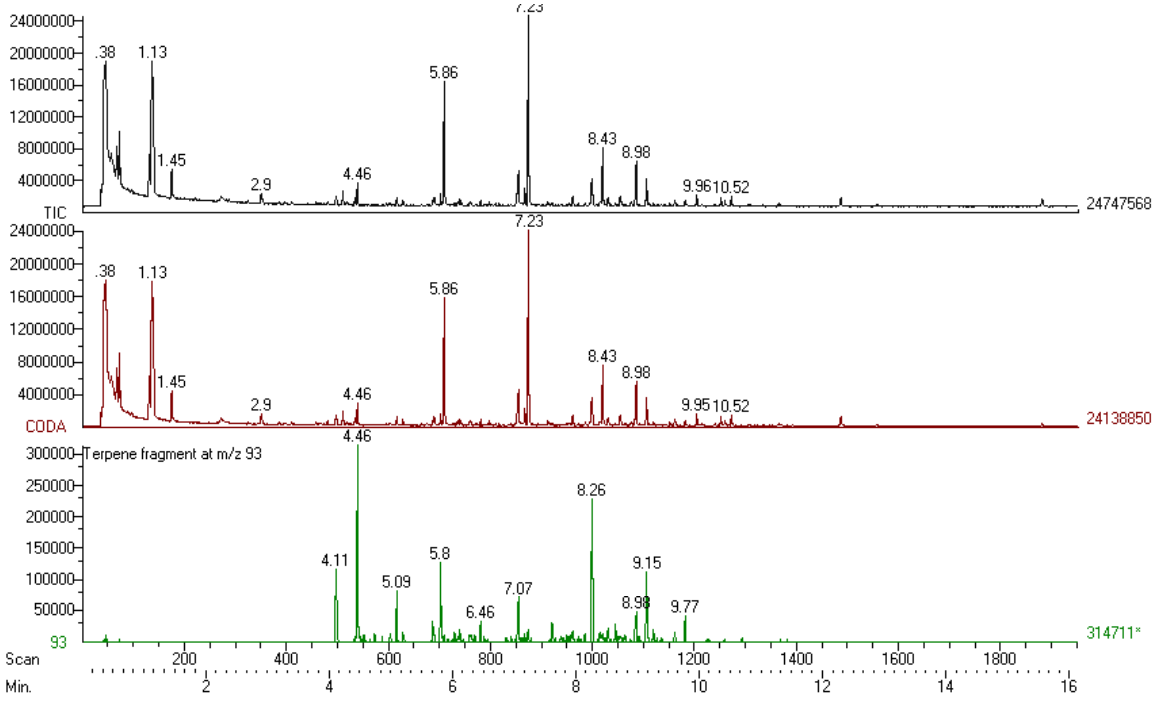


Figure 6. Total ion current (TIC) and CODA chromatograms for tequila sample and mass chromatogram for m/z 93 (indicative of terpenes).

Library search for ethyl octanoate

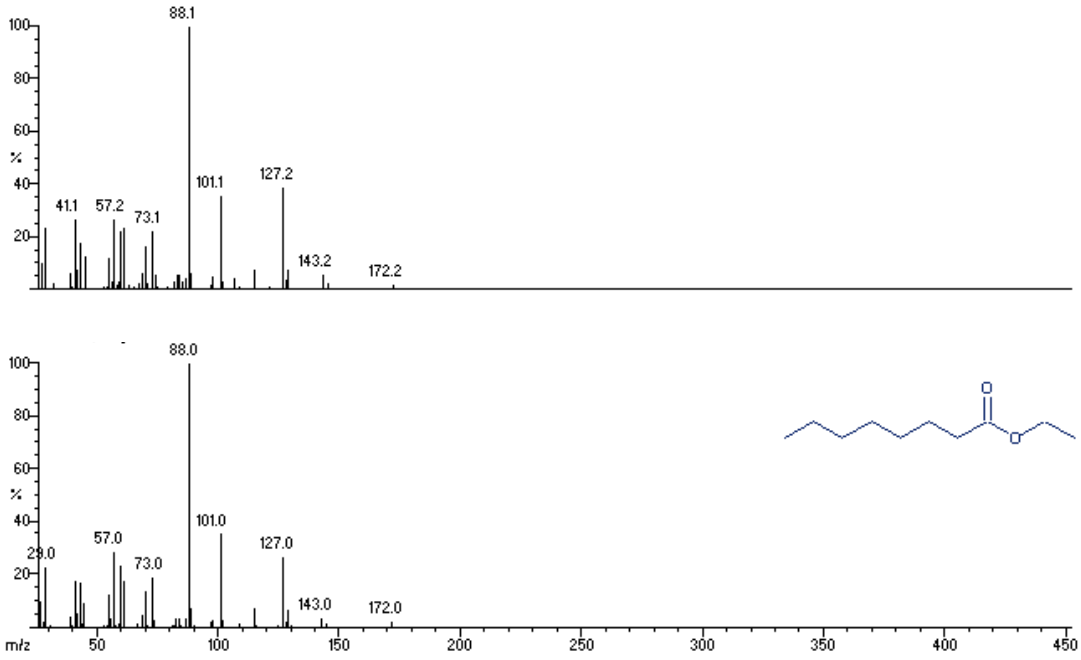


Figure 7. Mass spectrum and library database spectrum of ethyl octanoate.

Library search for linalool

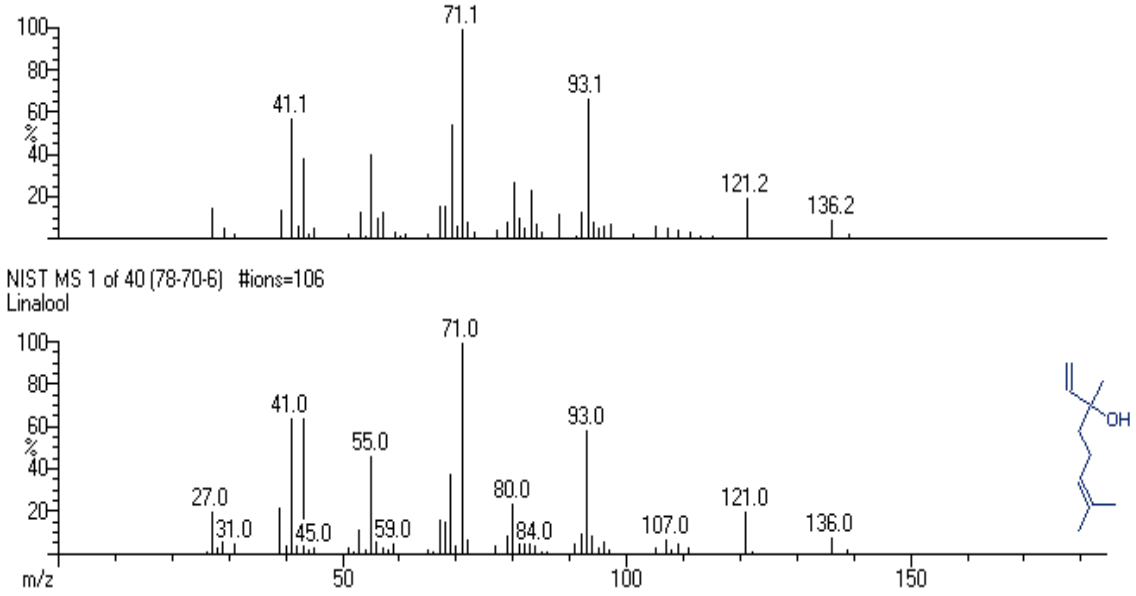


Figure 8. Mass spectrum and library database spectrum of linalool.

Conclusion: SPME provides a convenient method for sampling alcoholic beverages for GC/MS analysis. High-resolution mass spectrometry using the JEOL *GCmate II* provides the additional benefit of elemental composition determinations for confirming assignments, identifying unknowns, and making structural assignments for fragment ions.

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[1] TSS-2000 is a trademark of Shrader Analytical Laboratories (www.shraderlabs.com).

[2] W. Windig, J.M. Phalp, and A.W. Payne*; Analytical Chemistry,* Vol. 68, No. 20, p. 3602.

For more information on equipment used in the experiment, visit the following websites:

www.alltechweb.com
www.sigmaaldrich.com
www.agilent.com