An Examination of Automated Sampling Techniques of Whiskey Samples

Application Note

Food and Flavor

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Abstract

There are a large variety of volatile compounds that can be found in alcohol. These volatile compounds can add or deter from the flavor of your drink. Trained analysts can often detect the flavors in liquors by smelling and tasting a sample. However, in order to establish the quality and essence of a liquor sample, further testing is required. Gas Chromatography coupled with Mass Spectrometry (GC/MS) is an effective tool for the determination of the flavor compounds in an alcohol. Utilizing different sampling techniques, the respective efficiency in the determination of flavor compounds in whiskey samples will be investigated.

Introduction:

Whiskey has a very complex flavor profile and no two whiskeys are alike. Since alcohol is a volatile liquid, there are several options for the sampling and analysis of whiskey. For this application, a Gas Chromatograph/Mass Spectrometer (GC/MS) was utilized for the analysis of the whiskey samples. The GC was employed in order to separate the volatile components of the whiskey while the MS was utilized for the determination of the analytes in the samples. In order to sample the whiskey, the FLEX autosampler was used. The software and hardware of the FLEX enabled changing from one sampling technique to another an easy task.

The first sampling technique employed was static headspace. During static headspace, method parameters are optimized in order to lower the partition coefficient of the sample. Once the sample is at equilibrium, an aliquot of the headspace is taken from the sample vial and injected into the GC. The advantages of static headspace are that it's simple, reproducible and has less carryover problems than other sampling techniques. The disadvantages are lower sensitivity and the technique is only good for the more volatile analytes.

Solid Phase Micro Extraction (SPME) was the second sampling technique evaluated for this analysis. SPME involves using a coated fiber and exposing it to the headspace of the sample. The extraction coating varies with the analytes to be extracted. The sample is brought to equilibrium and the fiber is injected into the headspace of the sample for extraction. Headspace SPME can be used with multiple matrices without issue. However, SPME fibers need to be cleaned between sampling in order to limit carryover and as analytes are extracted from the headspace of the sample, it is more difficult to extract the heavier compounds.

The final sampling technique was Large Volume Injection (LVI). LVI uses Programmable Temperature Vaporization (PTV) at the GC inlet during the split injection. This allows the solvent to be eliminated using the purge flow to the split vent during injection. The analytes are retained on the inlet liner while the solvent is vented. Then, the inlet is heated in order to transfer the analytes onto the GC column. Thus, a large injection can be used in order to gain sensitivity without the worry of the sample matrix causing problems with the GC. This sampling allows for the detection of both the light and the heavy compounds in the sample. Moreover, there is no sample preparation or equilibration time.

Experimental:

The FLEX sampling system was set up to run with an Agilent 7890A GC and 5975 inert XL MS. A Restek Stabilwax DA 30m X 0.25mm X 0.25μm column was mounted in the GC for analyte separation. The FLEX system was configured with a 10μl syringe and the TITAN PTV LVI was installed in the GC for the LVI sampling. A 50/30μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) coated fiber was employed for headspace SPME and finally a 2.5ml headspace syringe was utilized for static headspace. The sampling and analysis parameters for all three techniques are listed in Tables 1 and 2 below.

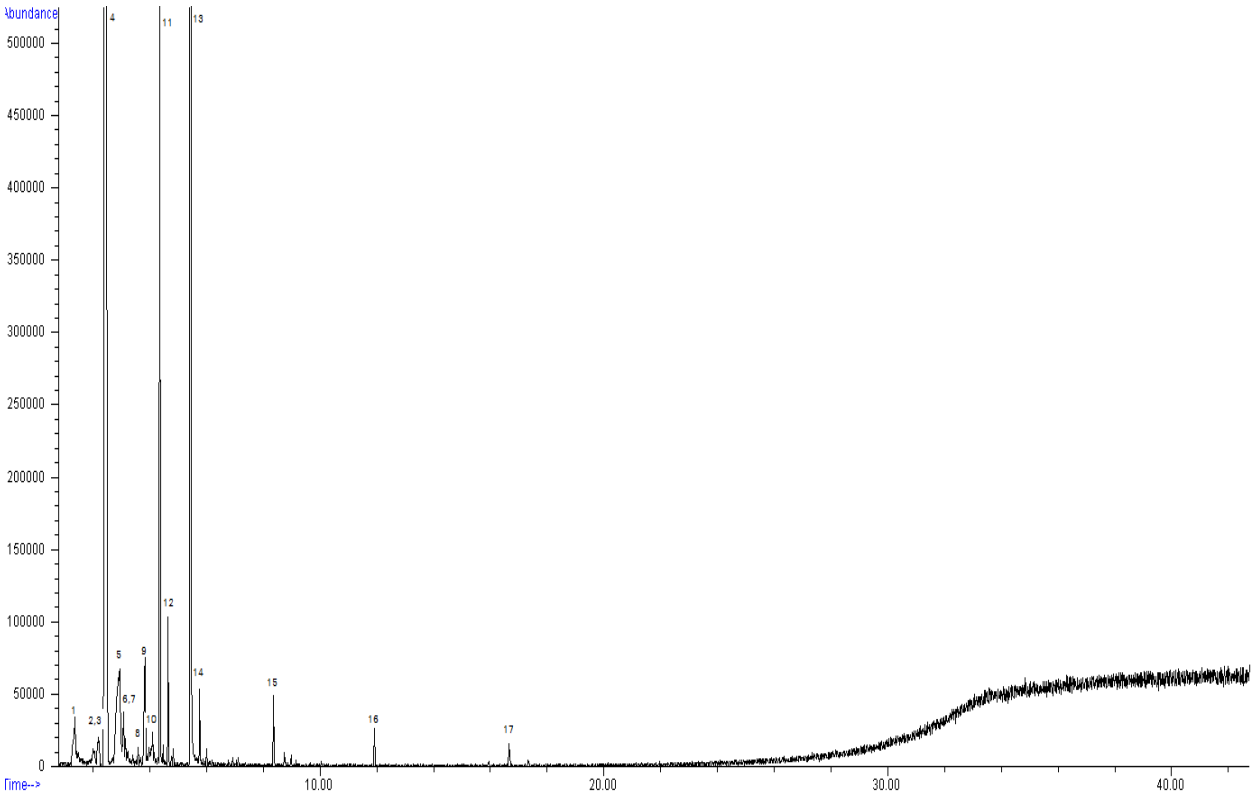
GC/MS Agilent 7890A/5975 inert XL			
Method Type	**Liquid**	**Headspace**	**SPME**
Inlet	PTV/Solvent Vent	Split/Splitless	Split/Splitless
Inlet Temp.	70°C hold for 0.7min, 200°C/min to 240°C hold for 42.2min	220°C	220°C
Inlet Head Pressure	11.809 psi	14.875 psi	11.809 psi
Mode	Solvent Vent	Split	Pulsed Splitless
Split Ratio	NA	40:1	NA
Purge Flow to Split Vent	50ml/min at 1.5min	NA	10ml/min at 2.01min
Vent Flow	100ml/min	NA	NA
Vent Pressure	2.5psi until 0.5min	NA	NA
Cryo	off	NA	NA
Injection Pulse Pressure	NA	NA	20psi until 2min
Inlet Liner	TITAN XL SB Deactivated Liner with Glass Wool	Restek Split Liner 1mm X 6.3 X 78.5	Restek SPME Liner 0.75mm X 6.35 X 78.5
Column	Restek Stabilwax®-DA 30m X 0.25mmID X 0.25µm df	Restek Stabilwax®-DA 30m X 0.25mmID X 0.25µm df	Restek Stabilwax®-DA 30m X 0.25mmID X 0.25µm df
Oven Temp. Program	45°C hold for 2 min, ramp 20°C/min to 100°C, hold for 0 min, ramp 5°C/min to 240°C, hold for 10min, 42.2 min total run time	45°C hold for 2 min, ramp 20°C/min to 100°C, hold for 0 min, ramp 5°C/min to 240°C, hold for 10min, 42.2 min total run time	45°C hold for 2 min, ramp 20°C/min to 100°C, hold for 0 min, ramp 5°C/min to 240°C, hold for 10min, 42.2 min total run time
Column Flow Rate	1.0mL/min	1.0mL/min	1.0mL/min
Gas	Helium	Helium	Helium
Total Flow	53mL/min	44ml/min	14ml/min
Source Temp.	230°C	230°C	230°C
Quad Temp.	150°C	150°C	150°C
MS Transfer Line Temp.	220°C	220°C	220°C
Scan Range	m/z 50-300	m/z 50-300	m/z 50-300
Scans	5.5 scans/sec	5.5 scans/sec	5.5 scans/sec
Solvent Delay	0.7min	0.7min	0.7min

Table 1: GC/MS Experimental Parameters

FLEX Autosampler			
General			
Method Type	Liquid	Headspace	SPME
GC Ready	Wait	Continue	Continue
GC Cycle Time	46min	46min	46min
Constant Heat Mode	NA	Yes	Yes
Rinse			
Rinse Volume	7µl	NA	NA
Number of Rinses	2	NA	NA
Fill Rate	50%	NA	NA
Dispense Rate	100%	NA	NA
Incubate Agitate			
Incubation Temperature	NA	60°C	NA
Incubation Time	NA	20.1min	NA
Agitation Speed	NA	80%	NA
Agitation Delay	NA	0.1min	NA
Agitation Time	NA	20min	NA
Incubate Stir			
Incubation Temperature	NA	NA	60°C
Incubation Time	NA	NA	20min
Stirrer Speed	NA	NA	Medium
Extraction			
Fiber Guide Depth	NA	NA	50%
Sample Vial Fiber Depth	NA	NA	1cm
Fiber Extraction Time	NA	NA	20min
Wait			
Wait Input	GC Ready	GC Ready	GC Ready
Air Volume Gap			
Air Fill Volume Percent	10% (1ul)	NA	NA
Sample Fill			
Syringe Temperature	NA	85°C	NA
Sample Volume	NA	80% (2000µl)	NA
Sample Fill Delay	NA	1sec	NA
Desorbtion			
Fiber Insertion Depth	NA	NA	1cm
Fiber Desorbtion Time	NA	NA	2min
Injection Start Input	NA	NA	Start
Injection			
Injection Rate	10%	5%	NA
Injection Volume	60% (6µl)	80% (2000µl)	NA
Rinse			
Rinse Volume	7µl	NA	NA
Number of Rinses	2	NA	NA
Fill Rate	50%	NA	NA
Dispense Rate	100%	NA	NA
Condition Fiber			
Fiber Temperature	NA	NA	250°C
Condition Time	NA	NA	5min

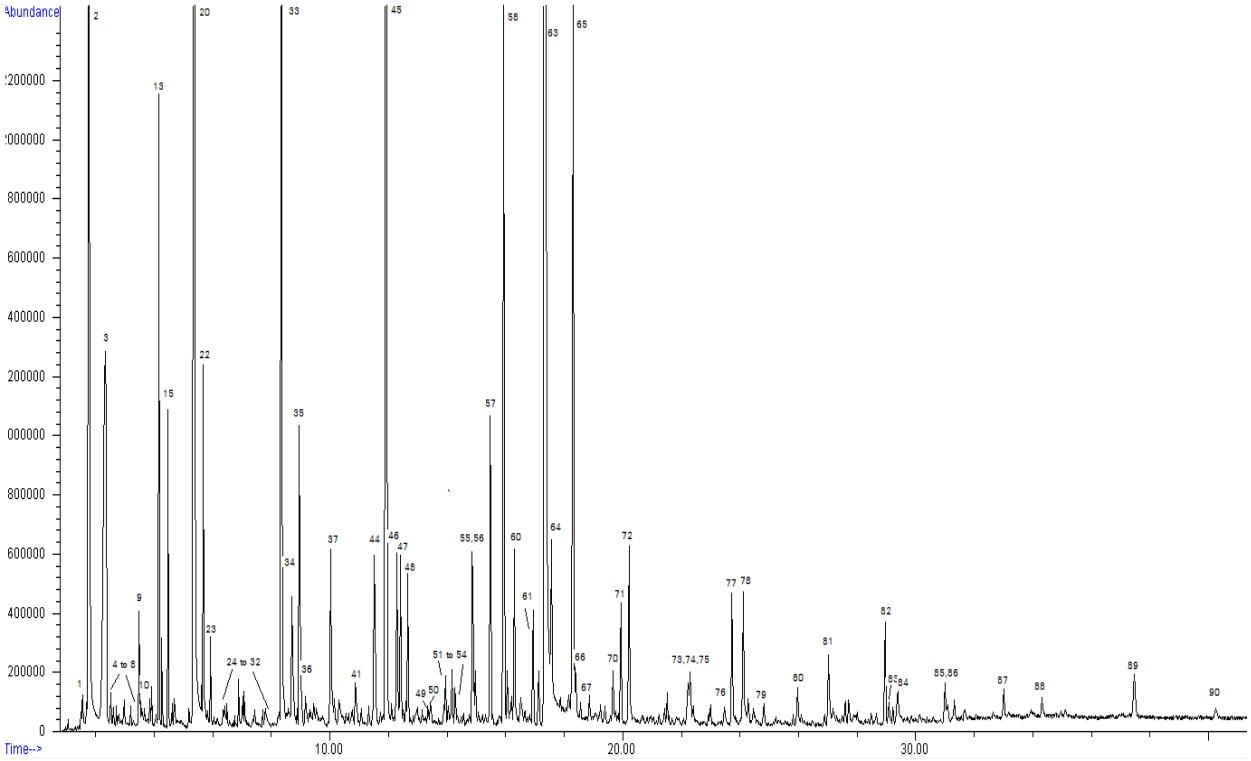
Table 2: FLEX Autosampler Experimental Parameters

In order to perform the static headspace sampling, 1g of sodium chloride was added to each sample vial along with 5 milliliters of whiskey. The samples were then sealed in a 20ml headspace vial and run as described previously. For SPME, again 5 milliliters of whiskey and 1g of sodium chloride were added to the sample vial and sealed. Several different fiber coatings were tried in order to establish the best fiber for the application and after the fiber was decided upon, the samples were run as described. Finally, 5μl was used for the LVI technique and no sample preparation was involved. Each sampling method was run four times in order to establish the reproducibility of the technique. Using the Agilent library software, a list of the resulting compounds was established. Chromatograms and analyte analysis are displayed in Figures 1 through 4.



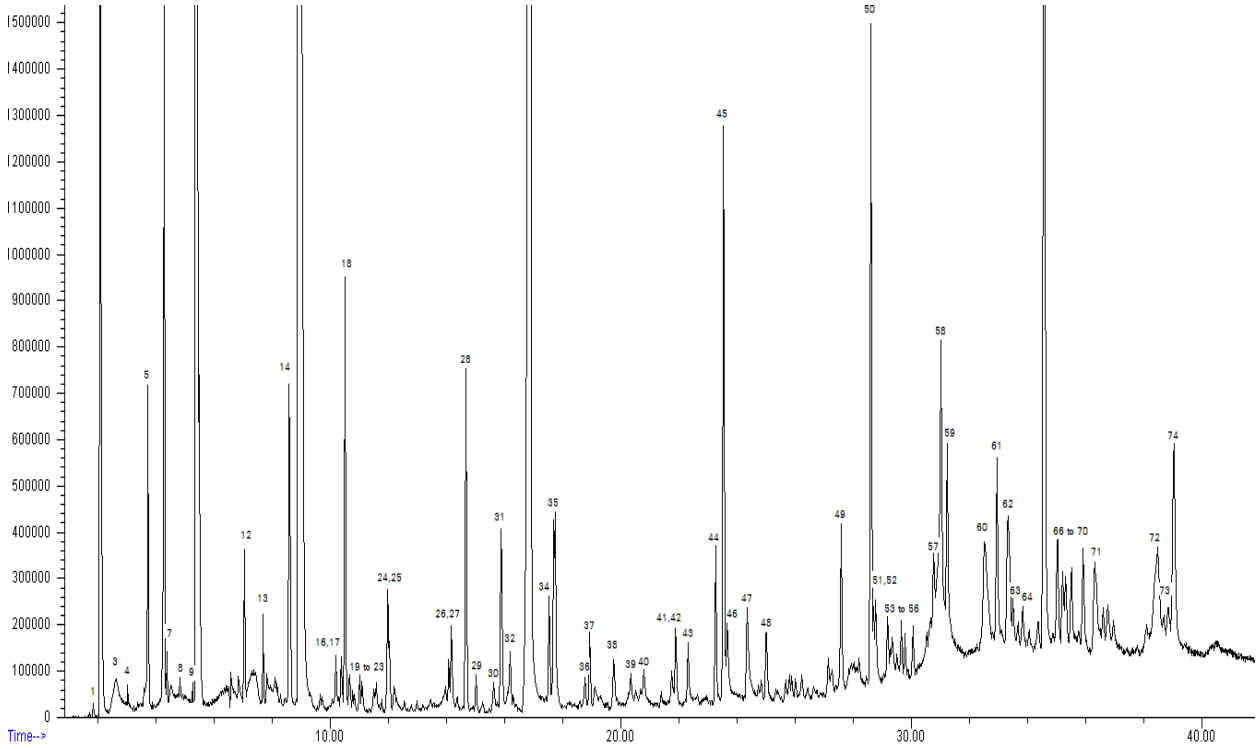
1	unknown	7	2-methyl propanoic acid ethyl ester	13	3-methyl-1-butanol
2	formic acid ethyl ester	8	2-methylpropyl ester acetic acid	14	hexanoic acid ethyl ester
3	diethoxymethane	9	2-fluoro-1-propene	15	octanoic acid ethyl ester
4	ethyl acetate	10	3-methyl butanoic acid ethyl ester	16	decanoic acid ethyl ester
5	2-methyl-2-butanol (?)	11	2-methyl-1-propanol	17	benzyl alcohol
6	propanoic acid ethyl ester	12	1-butanol, 3-methyl acetate		

Figure 1: Static Headspace Sampling at 60°C with 20 Minute Agitation



1	diethoxymethane	32	nonanal	63	phenyl ethyl alcohol
2	ethylacetate	33	octanoic acid ethyl ester	64	unknown
3	boric acid, triethyl ester	34	carbonyl sulfide	65	5-butyldihydro-4-methyl cis 2(3H)furanone
4	propanoic acid ethyl ester	35	2-furancarboxaldehyde	66	1-dodecanol
5	propanoic acid, 2-methylethyl ester	36	2-ethyl-1-hexanol	67	cyclododecane
6	propane, 1,1-diethoxy-2-methyl	37	nonanoic acid ethyl ester	68	phenol
7	1-butoxy-1-ethoxyethane	38	5-nonanol	69	unknown
8	acetic acid, 2-methyl propyl ester	39	butyl caprylate	70	d\ihydro-5-pentyl- 2(3H)furanone
9	unknown	40	1-octanol	71	tetradecanoic acid ethyl ester
10	butanoic acid, 2-methylethyl ester	41	5-methyl-2-furancarboxaldehyde	72	octanoic acid
11	butanoic acid, 3-methylethyl ester	42	hexadecane	73	eugenol
12	butane, 1,1-diethoxy-3-methyl	43	octamethyl trisiloxane	74	unknown
13	2-methyl-1-propanol	44	benzonitrile	75	4-ethyl phenol
14	1,1-ethoxy ethoxy pentane	45	decanoic acid ethyl ester	76	1,1-dimethylethyl-methyl benzene
15	3-methyl acetate-1-butanol	46	3-methylbutyl ester octanoic acid	77	hexanoic acid ethyl ester
16	ethyl ester pentanoic acid	47	ethyl cis-4-decanoate	78	decanoic acid
17	benzene ethanamine, N-pentafluorophenyl methylene (?)	48	butanedioic acid diethyl ester	79	2,6-bis(1,1-dimethylethyl)-phenol
18	1-butanol	49	tetradecanal	80	Cyclotetradecane
19	Dodecane	50	4-ethyl benzaldehyde	81	benzoic acid
20	3-methyl-1-butanol	51	undecanoic acid ethyl ester	82	unknown
21	1,1-diethyoxy hexane	52	3-methyl 2-butanoic acid	83	vanillin
22	hexanoic acid ethyl ester	53	acetone dimethyl hydrozone	84	1-octadecene
23	1,1-diethoxy-2-methyl propane	54	trans-1-butyl-2-methylcyclopropane	85	benzamide
24	tridecane	55	9-decen-1-ol	86	dibutyl phthalate
25	septum bleed	56	1,9-nonanediol	87	hexanoic acid bis(2-ethylhexyl ester)
26	1,1,3-triethoxy propane	57	acetic acid 2-phenyl ethyl ester	88	hexadecanoic acid
27	heptanolc acid ethyl ester	58	dodecanoic ethyl ester	89	2,6,10-dodecatrien-1-ol, 3,7,11 trimethyl
28	trimethyl silanol (?)	59	hexanoic acid	90	di-n-octyl phthalate
29	1-hexanol	60	3-methylbutyl decanoate		
30	3-ethoxy-1-propanol	61	trans-4-hydroxy-3-methyl octanoic acid lactone		
31	tetradecane	62	butanedioic acid diethyl ester		

Figure 2: SPME Sampling at 60°C with 20 Minutes of Stirring



1	diethoxymethane	26	3-methyl bicyclo[4.1.0]heptane	51	1-penten-3-ol (?)
2	ethylacetate	27	5-methyl-2-furanone	52	4-methyl-2,5-dimethoxybenzaldehyde
3	boric acid, triethyl ester	28	dodecanoic ethyl ester	53	2-methoxy-4-propyl phenol
4	unknown	29	3-methylbutyl decanoate	54	4-hydroxy-3-methoxy benzoic acid ethyl ester
5	2-fluoro-1-propene	30	hexanoic acid	55	1-(4-hydroyx-3-methoxyphenyl)-ethanone
6	2-methyl-1-propanol	31	2-methoxy phenol	56	4-hydroxy-3-methoxy benzeneacetic acid
7	1-butanol-3-methyl acetate	32	5-butyldihydro-4-methyl-cis-2(3H)furanone	57	2′,4′-dihydroxy-3′methylpropiophenone
8	1-butanol	33	phenyl ethyl alcohol	58	hexanoic acid
9	hexanoic acid ethyl ester	34	3-methyl-4-hydroxyoctanoic acid gamma lactone	59	2,6-dimethoxy-4-2-propenyl phenol
10	2-methyl-1-butanol	35	trans-4-hydroxy-3-methyloctanoic acid lactone	60	2,3,6-trimethyl-naphthalene
11	3-methyl-1-butanol	36	phenol	61	methyl-alpha-D-xylofuranoside
12	octanoic acid ethyl ester	37	tetradecanoic acid ethyl ester	62	unknown
13	N-nitrosodimethylamine (?)	38	octanoic acid	63	3,4,5-trimethoxy-benzenemethanol
14	2-furancarboxaldehyde	39	1-octen-3-ol	64	hexadecanoic acid
15	carbonyl sulfide	40	unknown	65	4-hydroxy-3,5dimethoxy benzaldehyde
16	propanoic acid	41	2-methoxy-4-(1-propenyl)-phenol	66	unknown
17	5-methyl-2-furancarboxaldehyde	42	unknown	67	4-hydroxy-3-methoxy benzene acetic acid
18	decanoic acid ethyl ester	43	1-(2-hydroxy-5-methylphenyl)-ethanone	68	unknown
19	4-cyclopentene-1,3-dione	44	hexadecanoic acid ethyl ester	69	1,(4-hydroxy-3,5-dimethoxyphenyl)-ethanone
20	unknown	45	2,6-dimethoxy phenol	70	1-(2,4,6-trihydroxy-3-methylphenyl)-1-butanone
21	ethyl cis-4-decenaote (?)	46	decanoic acid	71	unknown
22	unknown	47	glycerin	72	unknown
23	butanioic acid	48	eugenol	73	caffeine
24	butanedioc acid diethyl ester	49	5-(hydroxymethyl)-2-furancarboxaldehyde	74	3-methoxycinnamic acid
25	2-furanmethanol	50	vanillin		

Figure 3: LVI Sampling 5μl Injection

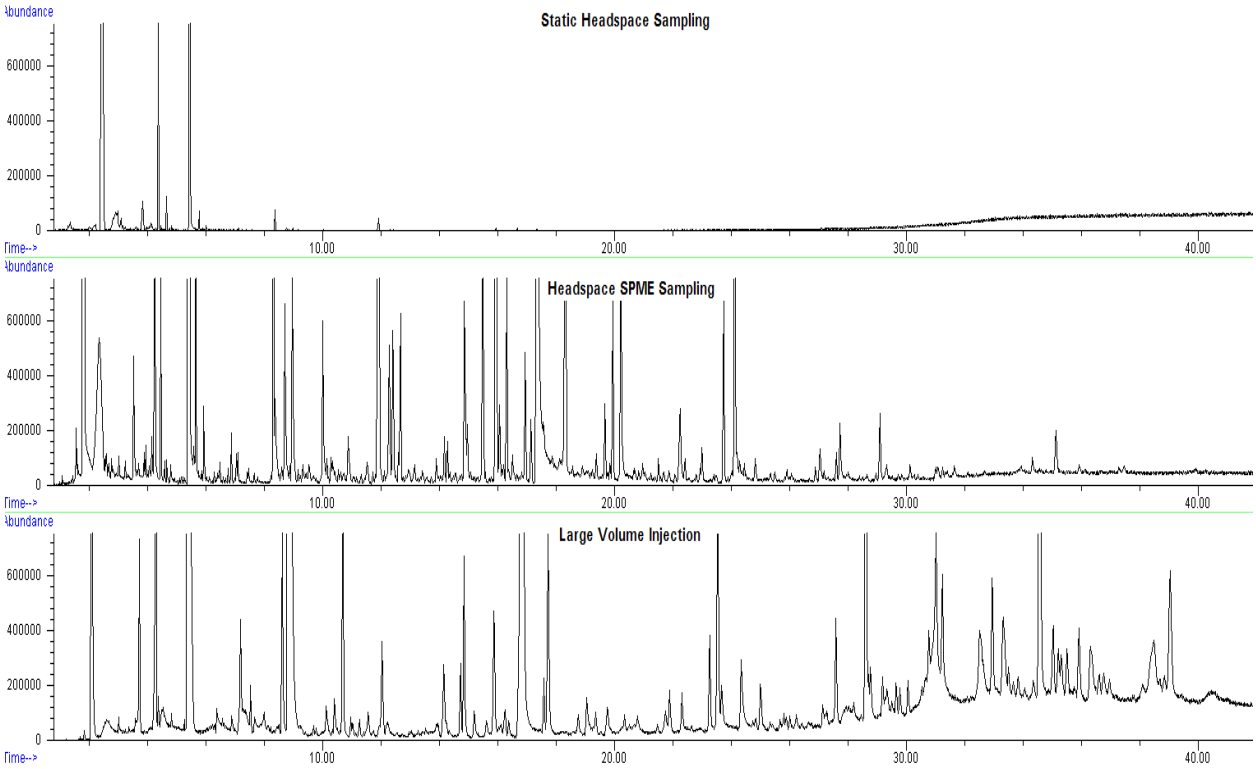


Figure 4: Overlay of the Three Different Sampling Technique Chromatograms

Conclusions:

The three sampling techniques were all reproducible and provided valuable results. The static headspace method was best for the more volatile compounds in the sample matrix. This type of sampling would not be good for the determination of the less volatile components, but would provide valuable information on the lighter compounds. SPME, on the other hand, proved to be an excellent way to determine both the lighter and the mid-range compounds in the whiskey. SPME had less sensitivity with the heaviest analytes; however, it did provide a lot of information and very sharp peaks for the determination of flavor compounds in the whiskey. LVI showed results over the entire analyte range from light to heavy. Conversely, the peaks were not as sharp and the chromatography was not as clean as static headspace and SPME. The FLEX autosampler proved to be an excellent system for whiskey sampling. Method development was easy with the innovative software and changing from headspace to SPME to LVI took minutes.

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