Characterization of Tequila by GC-TOFMS and Solid Phase Micro Extraction (SPME)

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Key Words: GC-TOFMS

1. Introduction

A number of tequilas were analyzed using a Fast Gas Chromatographic method combined with SPME in order to have a good understanding of the chemical composition of the tequila flavor. The tequilas were then compared to determine the similarities and differences.

In general, there has been relatively little information published with regards to the chemical composition of tequila flavor. One of the reasons is that the components that create the distinct flavor may not be distinguishable in larger quantities. In actuality the components that give tequila its distinct flavor qualities are present at very low levels and are not normally seen by GCMS alone. Efforts have been made to reconstitute the tequila flavor, however this has not been successful.[1] Combining SPME and GCMS techniques provides a simple, economical way to concentrate the small components in the tequila and identify them. Figure 1 is a typical chromatogram of tequila.

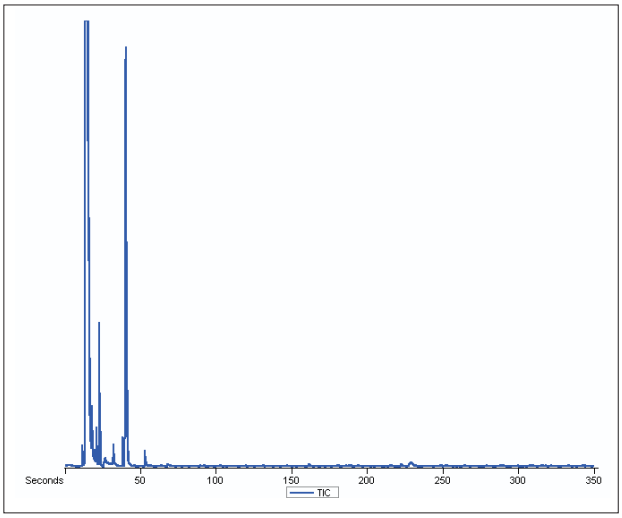

Figure 1. Chromatogram of Tequila using Fast Temperature Program.

2. Experimental Conditions

Materials and Methods

GC-Parameters: Agilent® 6890
Column: RTX-5®; 10 m x 0.18 mm x 0.20 µm
Injector Temp: 250°C
Split Flow: 10:1 or 100:1
Oven Program:
35°C for 0.5 minute, to 275°C at 70°C/minute, hold for 2.07 minutes
Flow Rate:
1.5 mL/minute Helium at constant flow
SPME Fiber:
2 cm-50/30 um DVB/Carboxen/PDMS

MS-Parameters: Pegasus II GC-TOFMS
Mass range: 35 to 450 amu
Acquisition rate: 50 spectra/second
Ion source Temp: 200°C
Total Acquisition Time: 360 seconds

In order to facilitate the comparison of the tequilas, a premium quality sample was first analyzed and set as a reference material to which the other tequilas would be compared. A 2 cm-50/30 um DVB/Carboxen/PDMS SPME fiber was prepared using the manufacturer's procedure. Four milliliters of tequila was placed in a 20 ml headspace vial and thermostated to 25°C. The SPME fiber was then exposed to the headspace for 10 minutes and desorbed at 250°C for 2 minutes. Figure 2 shows a chromatogram of the reference sample. The sample was then processed using a threshold noise of 50:1. Using these parameters, 113 peaks were found—a number of them at substantially high concentrations.

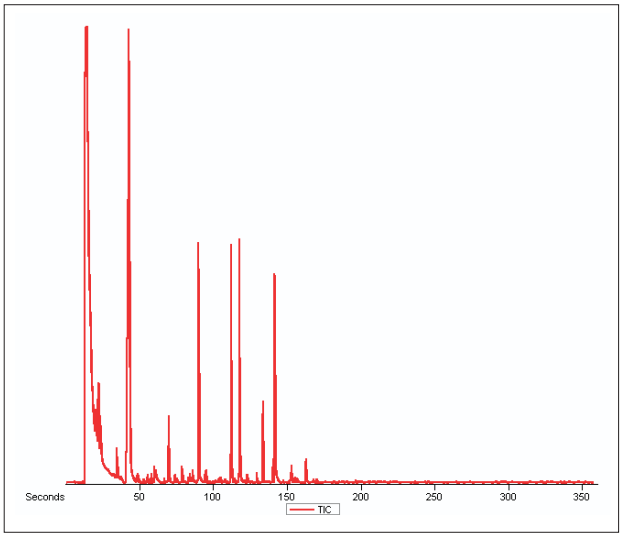

Figure 2. Total Ion Chromatogram (TIC) of Premium Tequila Desorbed from SPME Fiber.

Through the use of Processing algorithms, a substantial number of components were detected below the base line of the TIC or as coelutions. For most mass spectrometers this may present a problem since the peaks are found as protruding from the baseline and most chromatographic software is not written to detect coelutions. The rapid acquisition rate of the mass spectrometer and the high degree of spectral continuity makes it possible for the data processing software to find peaks below the base line of the TIC and deconvolute coelutions even when the coeluting components are present at a large concentration differential. Figures 3 and 4 are examples of coelutions in a tequila sample. Note that the peaks are less than 1 second wide and their apexes are separated by

less than 200 milliseconds. At an acquisition rate of 50 spectra/second there are sufficient data points for accurate profiling and deconvolution.

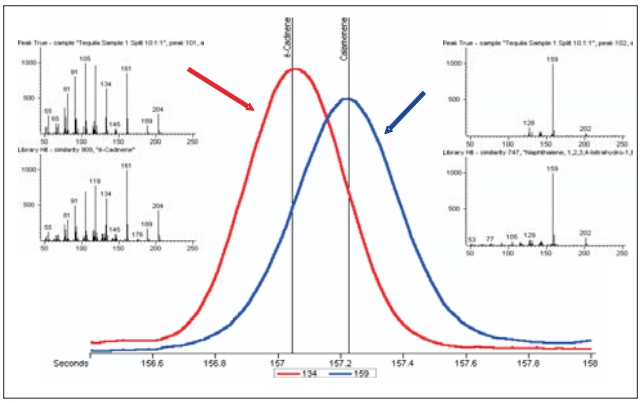

Figure 3. Coeluting Substances and their Deconvoluted Spectra.

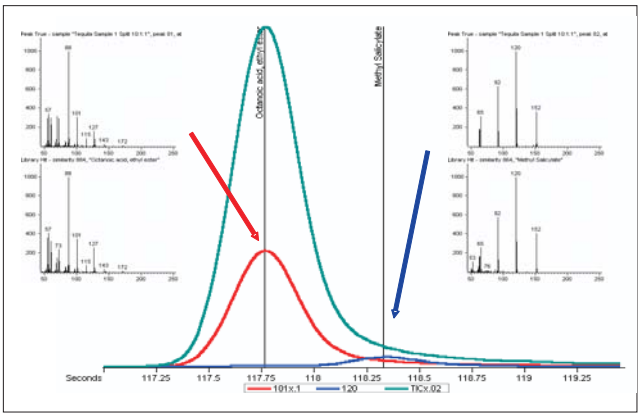

Figure 4. Coeluting Peaks and their Deconvoluted Spectra (Peaks are Detected Below the Baseline of the TIC).

3. Sample Comparisons

A sample is first analyzed and processed to find all its components and is then assigned as a reference to which all other samples will be compared. Once a reference has been assigned, each analyte is assigned a spectrum, a retention time (plus a deviation), and a signal-to-noise (S/N) tolerance. This means that when a sample is compared to the reference, an analyte will be found only if it matches the spectra, retention time, and S/N level. The Sample Comparison algorithm allows two samples to be compared to determine the components that are present in both samples and their relative concentrations (matches); the components present in the reference and not in the sample (not founds); and the components in the sample that are not present in the reference (contaminants). Analytes found outside a user-defined concentration window are also marked as a difference (out of tolerance) in the sample.

The premium tequila sample shown in Figure 2 was chosen as the reference. This sample displayed the characteristic golden color associated with a tequila that is brewed in wooden vats. A total of 113 analytes were identified in this sample.

Another tequila sample, also golden in color but from a different manufacturer, was analyzed and compared to the reference. Figure 5 shows the chromatograms of this sample superimposed on the reference. Notice the obvious differences and similarities between the two samples.

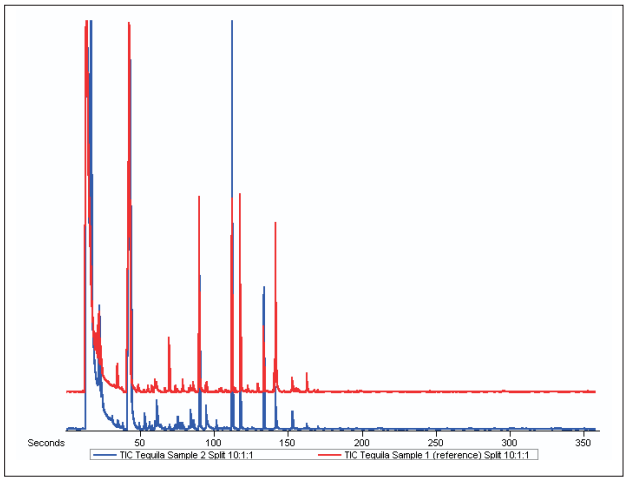

Figure 5. Comparison of Tequila Sample 2 (blue) to the Reference (red).

Tequila sample 2 was similar to the reference sample with the exception of the concentrations of the analytes. Of the 113 analytes present in the reference there were 28 of them that were not present in tequila sample 2. An example is shown in Figure 6. In this case, the analyte "Cadinene" is present in the reference but not in the sample.

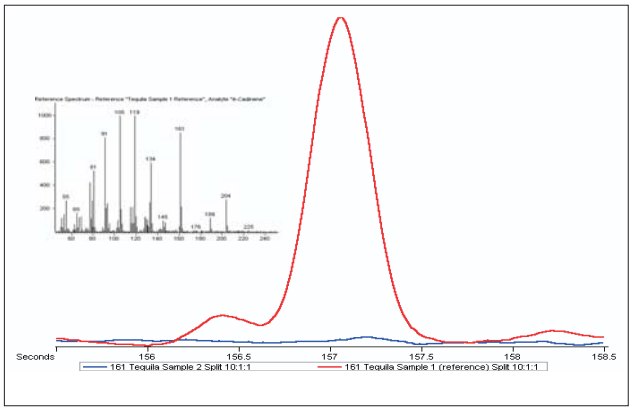

Figure 6. Cadinene is Present in the Reference (red) but Not in the Tequila Sample 2 (blue).

Tequila sample 3 also displayed a golden color and included a worm. This sample had a different smell than the reference, as well as a stronger taste (which is not necessarily better). The chromatogram for this sample (Figure 7) reveals a large number of analytes (contaminants) that were not present in the reference. The insert shows the section of the chromatogram between 100 and 115 seconds. Note that tequila sample 3 contains a number of analytes not present in the reference.

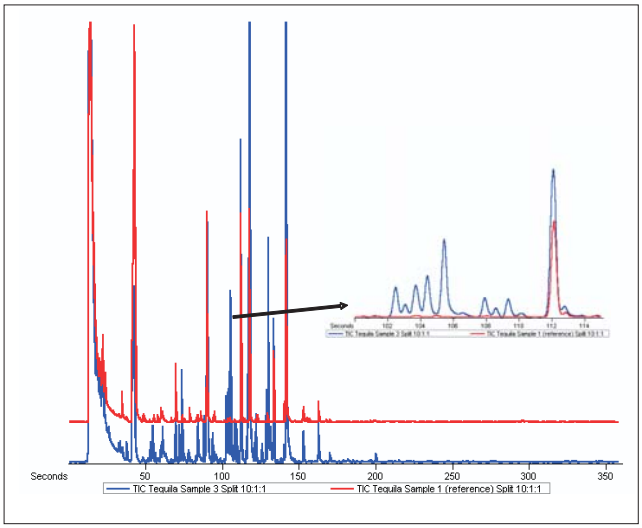

Figure 7. Comparison of Tequila Sample 3 (blue) to the Reference (red).

The tables below show the actual similarities and differences in the sample comparison. For example, Table 1 shows a partial list of analytes with relative concentrations found in both the reference and the sample. If one knows the actual concentrations of the analytes in the reference it is then possible to obtain real concentrations for the analytes in the comparison. Table 2 shows a partial list of analytes that were present in tequila sample 3, but were not present in the reference sample (contaminants).

Table 1. Comparison of Tequila Sample 3 to the Tequila Reference (Partial Table for Matching Analytes Showing Relative Concentrations).

Peak #	Name	R.T. (seconds)	Concentration	Match
5	Cyclotetrasiloxane, octamethyl-	89.993	186.15%	962
13	Benzene, 1-methyl-4-(1-methylethyl)-	94.273	226.38%	904
15	Limonene	95.013	83.71%	894
17	Benzene, 1,4-dichloro-	95.813	389.49%	787
48	Octanoic acid, methyl ester	108.173	341.54%	526
57	Cyclopentasiloxane, decamethyl-	112.113	163.33%	968
58	Benzene, 1,2,3,4-tetramethyl-	112.793	217.69%	943

Table 2. Comparison of Tequila Sample 3 to Tequila Reference (Partial table for "Contaminant" Analytes).

Peak #	Name	R.T. (seconds)	Similarity	Reverse
6	Octanal	90.573	963	978
11	1H-Tetrazol-5-amine	93.733	764	969
32	2-Pentene, 1-ethoxy-4-methyl-, (Z)-	102.513	737	777
33	6-Heptenoic acid, ethyl ester	103.093	883	887
36	Hexane, 1,1-diethoxy-	103.753	811	868
37	1,6-Octadien-3-ol, 3,7-dimethyl-	104.453	897	897
38	Benzaldehyde, ethenyl-	105.193	693	872
39	Nonanal	105.473	861	895
46	Phenylethyl Alcohol	107.473	783	854
47	2-Pentene, 1-ethoxy-4-methyl-, (Z)-	107.933	733	775
51	1,3-Dioxolane, 2-(2-propenyl)-	109.373	815	837

4. Conclusions

The described work demonstrates the use of GC-TOFMS and SPME to characterize and identify the components responsible for the aroma and taste of a number of tequila samples. The use of a Time-of-Flight mass spectrometer in this work is an innovative approach which demonstrates a number of advantages over other types of mass spectrometers.

The tequila comparisons were easily accomplished using the Automatic Comparison algorithm in the Pegasus software. The SPME technique made it is possible to gather low concentration analytes present in the tequilas and then desorb them for detection. One obvious conclusion is that each tequila manufacturer imparts different flavor or aromas depending on the manufacturing process. These differences can be easily quantified by using a comparison such as the one illustrated in this application note.

The strength of the Pegasus GC-TOFMS for the analysis of these complex mixtures lies in its automated data handling capabilities. Peak finding, spectral determination (deconvolution), library searching, and comparisons are all automatic, even when peaks are below the baseline of the TIC. This is possible due to the high degree of spectral continuity generated as well as the large data density allowed by the Pegasus GC-TOFMS system—up to 500 full mass spectra/second.

5. References

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