Statistical Differentiation of Baijiu Spirits Using SPME-GC-TOFMS and *ChromaTOF* Sync Software

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Introduction

Baijiu, a transparent distilled spirit regarded as China's national liquor, is one of the world's most consumed spirits. The manufacturing process of Baijiu is relatively complex—as with whisky, brandy and other distilled alcoholic beverages, several steps are involved, including fermentation, distillation, and aging. The diverse raw materials used, complexity of microorganisms used for fermentation, and different regional environments greatly contribute to the variety of Baijiu products and their aroma profiles. The characterization and differentiation of Baijiu samples according to their aroma-type and origin (region) can be conducted by gas chromatography and mass spectrometry (GC-MS). However, accurate analysis of these samples is challenging due to the high number and varying range of concentrations of compounds present, with detection and identification of trace level compounds being especially difficult.

Time-of-Flight Mass Spectrometry (TOFMS) detection improves this task due to the ability to collect full mass range, unskewed MS data with fast acquisition rates.

This application note describes the development of a headspace solid-phase microextraction (HS-SPME) method in combination with GC and LECO's *Pegasus* BT time-of-flight mass spectrometer (TOFMS) for the analysis of Baijiu's volatile organic components (VOCs).

HS-SPME was used to extract the VOCs of 10 Baijiu samples of different origin, for analysis by GC-TOF-MS. The comparison of the aroma profiles and the data analysis was facilitated using a new software tool—*ChromaTOF* Sync—which is a data processing tool that compiles peak information through sets of samples by performing automated peak finding and deconvolution of the high quality TOF-MS data on the set of samples. A composite sample set peak table is produced, and individual analyte trends through the sample set and overall sample trends can be observed and determined, providing the base for non-supervised statistical analysis. In this study, group type separation according to their respective class in terms of aroma-type and origin was obtained and statistically significant differences were easily highlighted.
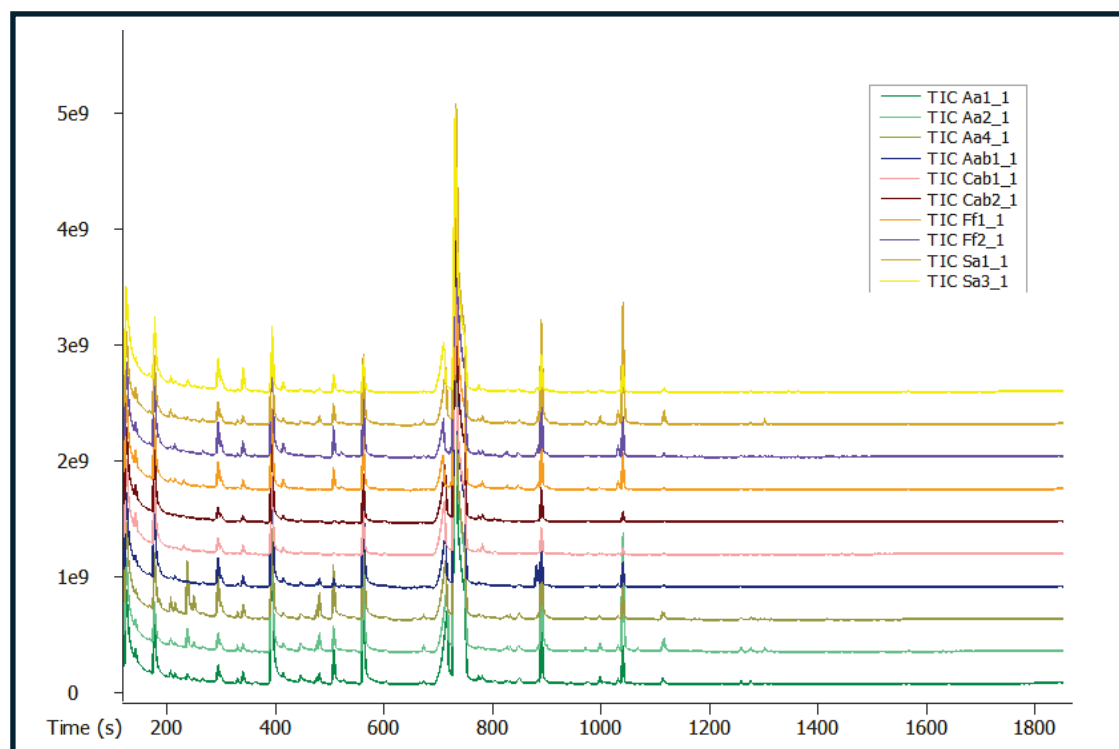

Figure 1: GC-MS chromatograms of the 10 Baijiu samples.

Experimental

Baijiu samples were diluted to 10% (v/v) of ethanol concentration using distilled water and subsequently 1 mL of each diluted Baijiu sample was transferred to a 20 mL headspace vial. Table 1 summarizes the parameters applied for HS-SPME incubation, extraction, and desorption in accordance with [1] as well as the GC-TOFMS instrumental parameters adopted. An n-alkane standard (C7-C30) was analyzed for calculation of linear retention indices (RIs). Table 2 lists the analyzed Baijiu samples with the corresponding aroma-type and origin.

Table 1: VOC sampling and analysis parameters.

	HS-SPME
SPME Fiber (2 cm)	50/30 µm coated with Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS)
Extraction Time	30 min at 50 °C
Desorption Time	3 min
Split Ratio	1:50
GC	**Agilent 7890**
Inlet	250 °C
Carrier Gas	He 1.4 mL/min
Column	Rxi-5MS 30 m x 0.25 mm i.d. x 0.25 µm coating
Oven Program	40 °C; ramp: 6 °C/min to 200 °C; 25 °C/min to 235 °C (5 min)
Transfer Line	280 °C
MS	**LECO *Pegasus* BT**
Ion Source Temp	250 °C
Mass Range	33 – 330 m/z
Acquisition Rate	10 spectra/s

Table 2: Aroma-type and origin of the investigated Baijiu samples.

Name	Type	Region
Aa1	Strong	Sichuan
Aa2	Strong	Sichuan
Aa4	Strong	Sichuan
Aab1	Jiang	Sichuan
Cab1	Jiang	Heilongijang
Cab2	Jiang	Heilongijang
Ff1	Feng	Shanxi
Ff2	Feng	Shanxi
Sa1	Strong	Jiangsu
Sa3	Strong	Jiangsu

Results

Figure 1 shows representative chromatograms for the ten Baijiu samples. Typically, the alignment and comparison of multiple chromatograms displays a major challenge in GC-MS data processing. Here it was possible to find similarities and differences among the analyzed Baijiu samples quickly and easily, by applying the new *ChromaTOF* Sync differentiation software tool.
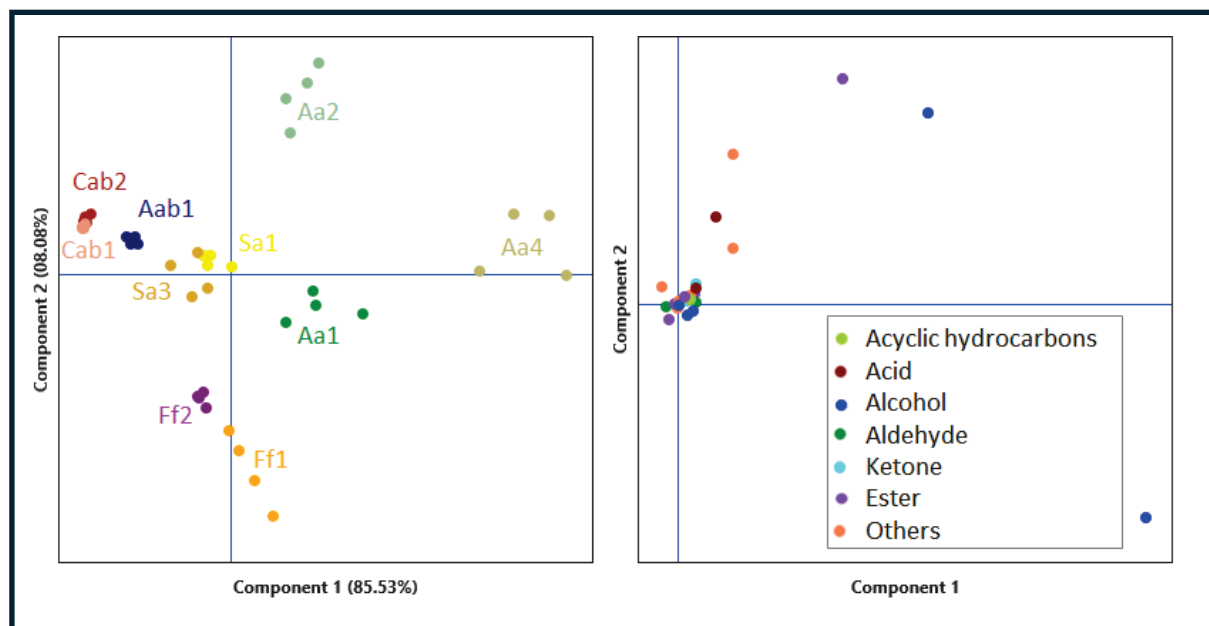
Figure 2: Principal Component Analysis (PCA) scores plot (left) displaying clustering of the Baijiu samples according to their aroma-type and origin. Loadings plot (right) with most marked distinguishing components color-labeled according to their chemical class.

Although multiple ways of displaying the results of differentiative analysis can be employed, one of the most used is the Principal Component Analysis (PCA) plot. It provides clustering of samples based on their overall similarity and trends. Figure 2 (left) shows the PCA scores obtained for this work.

The contribution of each feature to the sample grouping is summarized in the loadings plot as shown in Figure 2 (right). The displayed features were the most distinguishing components, and their identification was performed by comparison of deconvoluted mass spectra within *ChromaTOF* Sync using NIST MS libraries and RI calculation. In the figure, the components are assigned to their chemical class and encoded via colored labels.

The following compounds are examples of distinguishing components.

(A). The profile and trend of one of the most distinguishing features is shown in detail (Figure 3). The mass spectral comparison with the NIST MS library provides information for the compounds' identification suggesting 2-hexenoic acid, ethyl ester. The similarity match was 955/1000 with a RI difference of 4 RI units. This analyte was mainly observed in sample classes Aa1, Cab1, and Cab2, while it was present in lower abundance in the other samples, yet least detected in Aa2 and Aa4. The odor of this compound is described as rum-like, fruity, green, and sweet with a juicy undertone.

(B) The prominence of another statistically meaningful compound and its variability among the samples is displayed (Figure 4). It is showing the highest presence in the samples Aa and lowest presence in the samples belonging to Ff. The observed MS spectrum (bottom left of Figure 4) was matched to furfural (CAS: 98-01-1) in the NIST library database with a similarity score of 956/1000. This identification was further supported with RI information. The calculated RI for this peak was 835, showing good agreement with the library RI value of 833. Its aroma is described as sweet, woody, bready, and caramel-like with phenolic nuances.

(C) A compound identified as 1-hexanol (CAS: 111-27-3) with a similarity of 886 supported by RI information (calculated RI of 871 and library RI of 868) was detected with significantly higher abundance in the samples Aa, Ff, and Sa, compared with a lower level in the sample Aab, and lower presence still in the samples Cab (Figure 5). 1-Hexanol belongs to the green-herbal odor type and is described as ethereal, fusel, oily, fruity, alcoholic, sweet, and green.
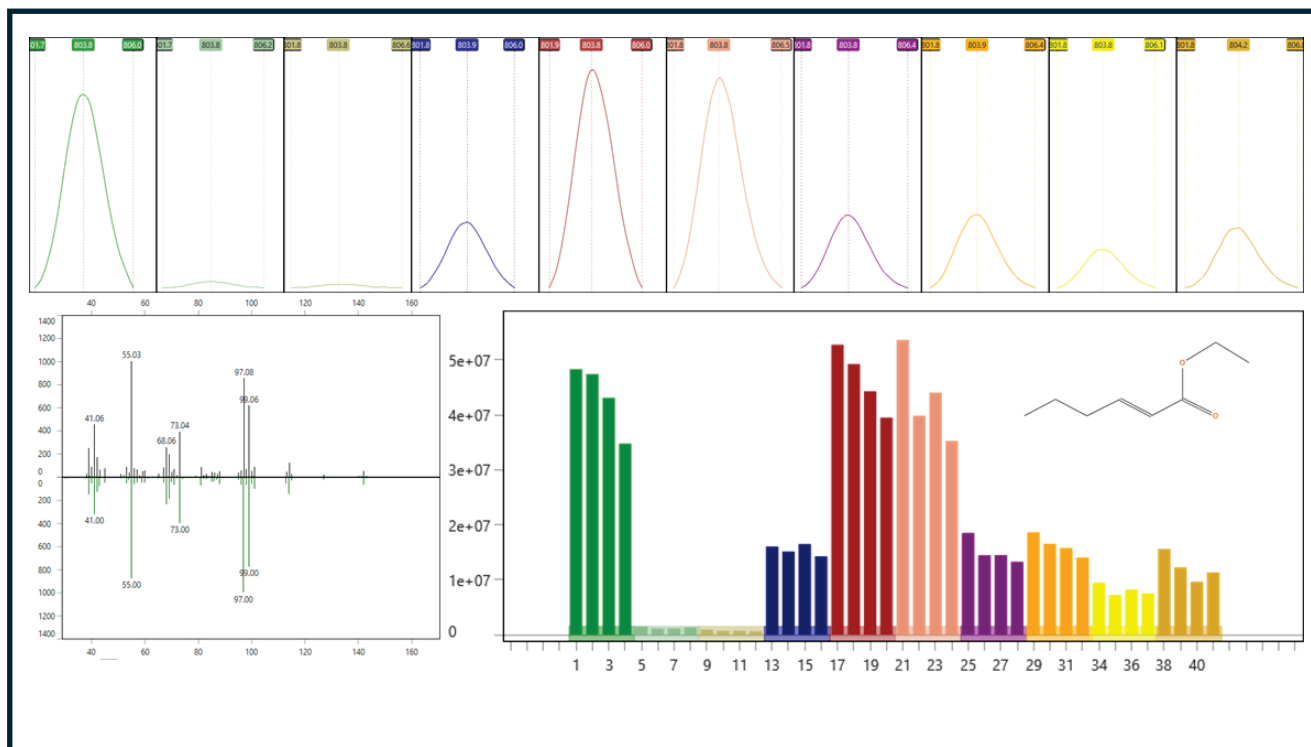
Figure 3: Representative example of chromatographic side-by side profile, mass spectral comparison and bar chart of 2-hexenoic acid, ethyl ester (CAS: 1552-67-6, RI: 1041) in all analyzed Baijiu samples. The sample area bars are compared here at m/z 97.
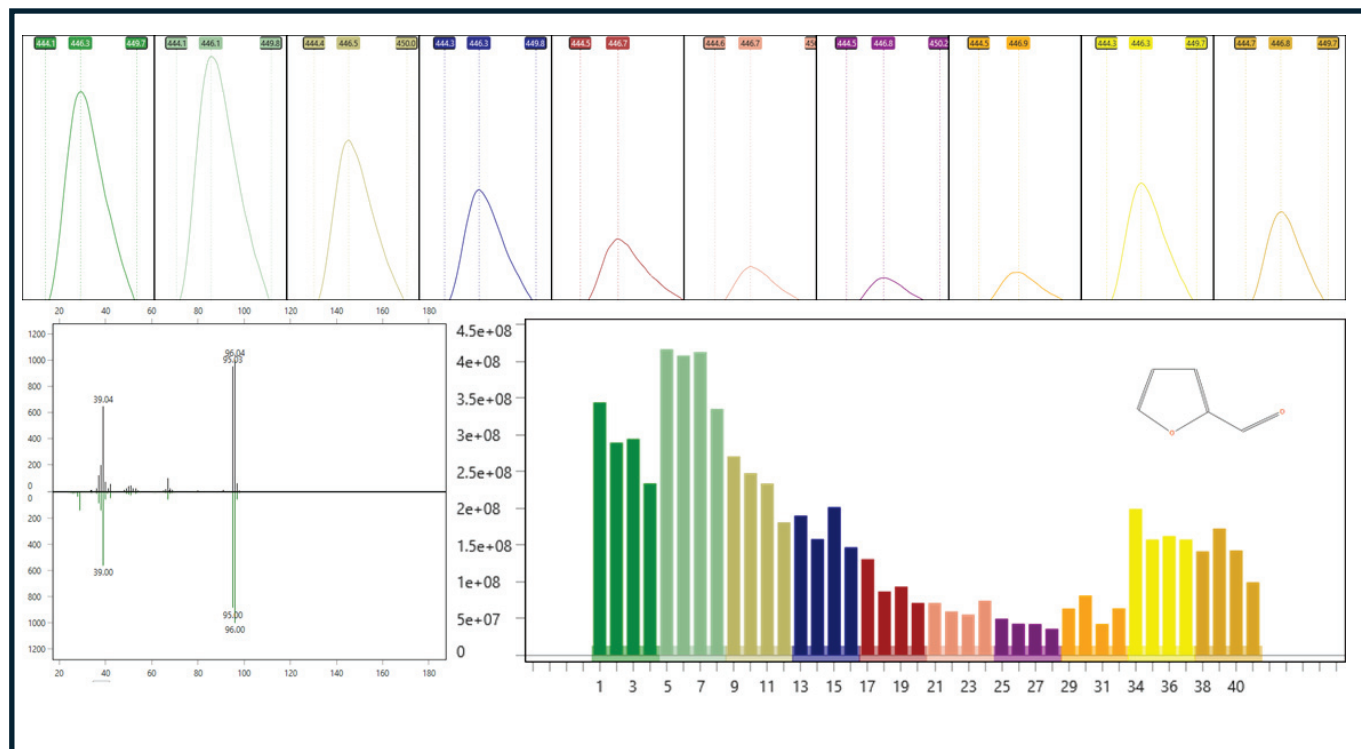

Figure 4: Representative example of chromatographic side-by side profile, mass spectral comparison and bar chart of furfural (CAS: 98-01-1, RI: 833) in all analyzed Baijiu samples. The sample area bars are compared here at m/z 95.
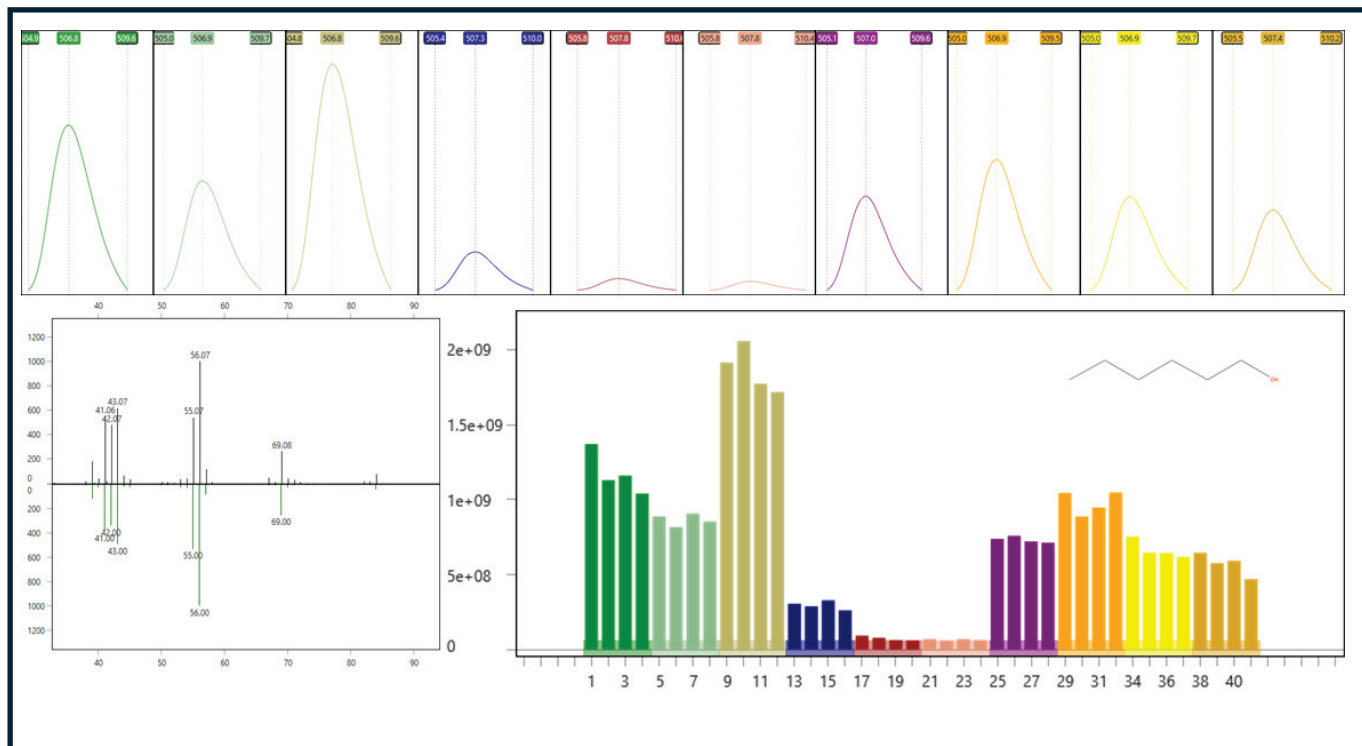
Figure 5: Representative example of chromatographic side-by side profile, mass spectral comparison and bar chart of 1-hexanol (CAS: 111-27-3, RI: 868) distribution in all analyzed Baijiu samples. The sample area bars are compared here at m/z 56.

Conclusion

HS-SPME combined with GC-TOFMS was used to analyze the VOCs in the headspace of 10 different Baijiu spirit samples. Understanding similarities and differences in the chemical composition and the associated aroma profiles of various Baijiu styles is a valuable addition to the quality assurance of different Baijiu types. This application note demonstrates a straightforward and effective approach to explore and interpret results obtained from such a rich data set, generated using LECO`s *Pegasus* BT GC-TOF-MS technology. Differentiating compounds such as 2-hexenoic acid, ethyl ester, and furfural were discovered easily by the means of *ChromaTOF* Sync Software. Compounds of interest were identified based on comparison of mass spectral information within *ChromaTOF* Sync and NIST MS libraries and retention indices calculations. The relative abundance of these features can be easily visualized e.g., in form of a bar chart (see Figure 3-5). The proposed workflow can be utilized to explore aroma profiles and follow individual analyte trends through the analyzed samples as well as overall sample trends within the tested sample set.

References

[1] He, X., & Jelen, H. H. (2021). Comprehensive two–dimensional gas chromatography–time of flight mass spectrometry (GC×GC–TOFMS) in conventional and reversed column configuration for the investigation of Baijiu aroma types and regional origin. Journal of Chromatography A, 1636, 461774.