Application Note

Instrument: Pegasus® BT 4D

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Statistical Differentiation of Baijiu Spirits Using SPME-GCxGC-TOFMS and ChromaTOF® Tile Software

Key Words: 2D GC; GCxGC; TOFMS; SPME; Tile; Aroma; Profiling; Alcoholic Beverage; Liquor

Introduction

Baijiu is a transparent distilled spirit, and it is regarded as China's national liquor. It's one of the world's most consumed alcohols. 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, 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 (i.e., production site or region) can be conducted by gas chromatography and mass spectrometry (GC-MS) analysis of their volatile organic compound (VOC) profile. However, accurate analysis of these samples is challenging due to the high number of compounds and varying range of concentrations present, with detection and identification of trace level compounds being especially difficult.

For the extensive investigation and aroma profiling of such rich samples, the use of comprehensive two-dimensional gas chromatography (GCxGC) can help to resolve the complex mélange of compounds thanks to its significantly greater separation power. Time-of-Flight Mass Spectrometry detection (TOFMS) improves this task due to the ability to collect full mass range data at sufficiently fast acquisition rates. This collection of such rich high-quality data allows effective use of deconvolution algorithms for automated peak finding (as embedded in the *ChromaTOF* software used here), which provides enhanced analyte characterization capability.

Herein, headspace solid-phase microextraction (HS-SPME) is used for the extraction of VOCs from 10 Baijiu samples of different origin (see Table 1) prior to GCxGC-TOFMS analysis. The comparison of the aroma profiles and the data analysis was facilitated by *ChromaTOF* Tile—a GCxGC statistical data processing tool. Supervised multivariate statistical analysis facilitated group-type separation and feature determination for analytes which are significantly different in the tested sample set. The presented workflow provides an easy and straightforward approach to acquire and interpret data for non-targeted GCxGC-TOFMS-analyses.

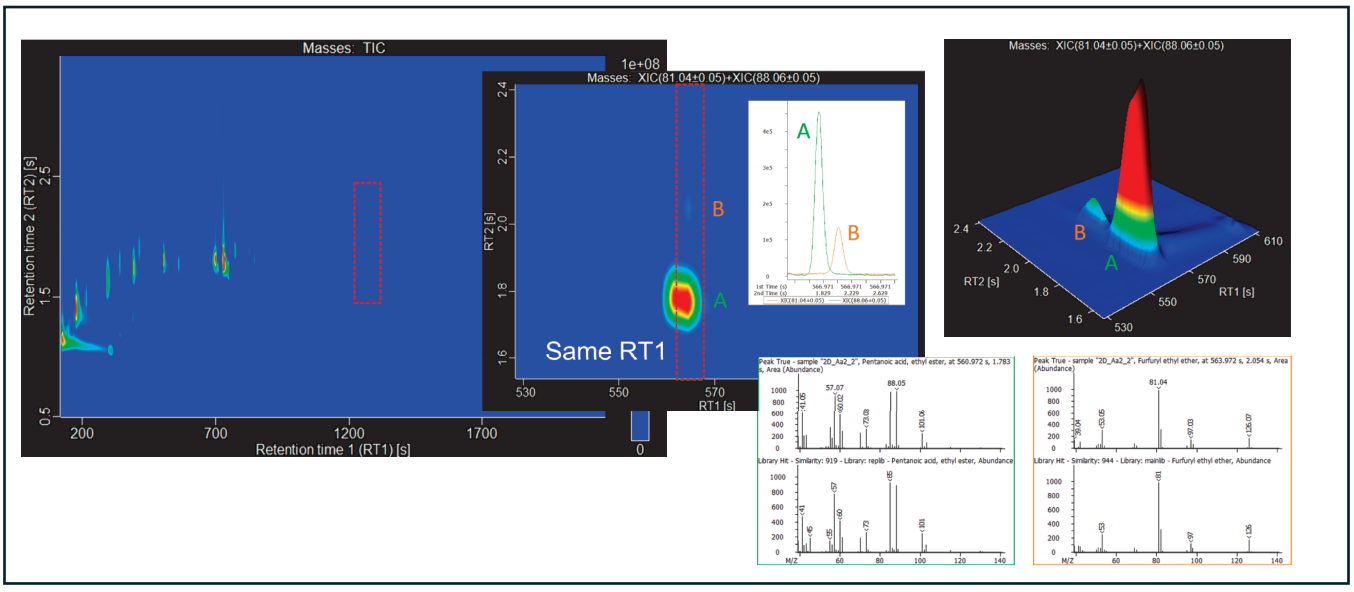

Figure 1: GCxGC contour plot of a representative Baijiu sample. An area with two compounds having the same RT1 is highlighted using the extracted ion chromatogram (XIC with m/z= 81.04 and 88.06). The zoomed-in area using the XIC is also displayed as 3D-plot on the righthand side. The compounds A and B were identified, based on the NIST MS library comparison with RI support, as pentanoic acid, ethyl ester and furfuryl ethyl ether, respectively.

Table 1: Aroma-type and origin of 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

Experimental

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

Table 2: VOC sampling and GCxGC-analysis parameters.

	HS-SPME
SPME Fiber (2 cm)	50/30 µm coated DVB/CAR/PDMS
Extraction Time	30 min at 50 °C
Desorption Time	3 min
Split Ratio	1:50
GCxGC	**Agilent 8890**
Injector	250 °C
Carrier Gas	He 1.4 mL/min
Columns	^{1}D: Rxi-5ms 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek) ^{2}D: Rxi-17Sil MS 0.9 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Modulation Period	3 s
Oven Program	40 °C; ramp: 6 °C/min to 200 °C; 25 °C/min to 235 °C (5 min)
Secondary Oven Temp	+5 °C (relative to the GC oven temperature)
Modulator Temp	+15 °C (relative to the secondary oven temperature)
Transfer Line	280 °C
TOFMS	**LECO *Pegasus* BT**
Ion Source Temp	250 °C
Mass Range	33 to 330 m/z
Acquisition Rate	200 spectra/s

Results and Discussion

Figure 1 shows exemplarily a contour plot of one of the ten Baijiu samples. The zoomed-in area shows two peaks with the same retention time (RT) in the first dimension. In fact, in a classical one-dimensional GC run, these compounds would coelute; however, due to the separation in the second dimension the two compounds are chromatographically resolved, enabling improved identification.

Typically, the alignment and comparison of multiple chromatograms pose a major challenge in GCxGC data processing. Here it was possible to determine analyte trends and differences among the analyzed Baijiu samples quickly and easily by applying the *ChromaTOF* Tile statistical analysis software. Non-targeted analysis of larger sample sets including replicates and the visualization of such data sets still pose challenges for analytical chemists. Therefore, Principal Component Analysis (PCA) is often used as one tool to reduce the dimensionality of data. Furthermore, it can display trends within the data set in form of clustering based on their chemical similarities and differences (Figure 2).

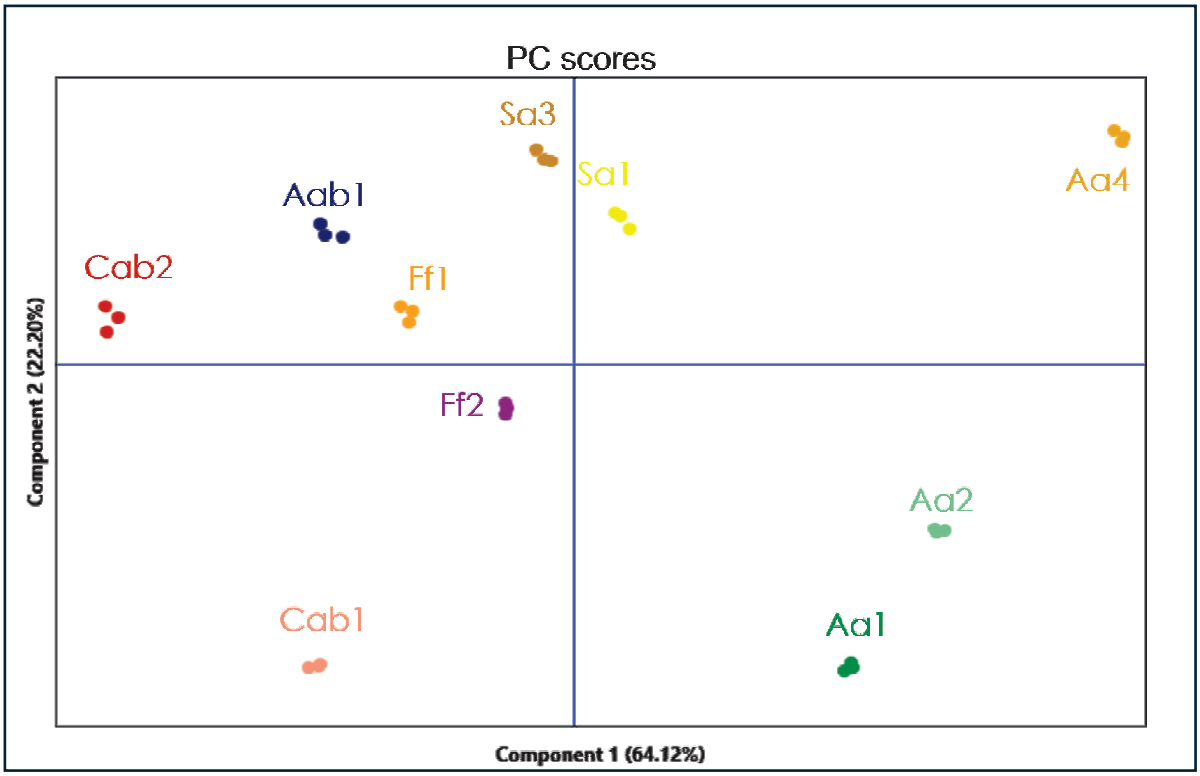

Figure 2: Principal Component Analysis (PCA) plot of all analyzed baijiu samples.

ChromaTOF Tile uses the Tile-based Fisher-Ratio approach, developed by Prof. Synovec, University of Washington, permitting identification of features which are different among the investigated classes. Multiple GCxGC-TOFMS data were explored in a fast and user-friendly way using normalized mass traces for area calculation. The following table (Figure 3) shows a snapshot of the software with a list of features with high Fisher-Ratio values indicating that there are substantial differences among the 10 tested Baijiu samples.

F-ratio hits | Chemical features | Summary

Name	Quant mass	R.I. calc	R.I. lib	R.I. Δ	Classe	1	2	3	4	5	6	7	8	9
2-Pentanone	67	689	685	4	Ketone	2225371.20	2307716.16	2474091.19	8219929.09	8281702.27	8074027.32	22953052.36	22730408.33	22539785.64
2-Pentanol	55	697	703	-6	Alcohol	64552843.42	66072489.42	75324285.21	126723295.44	122874585.88	137403903.68	429335063.77	420644660.85	447768550.90
Butanoic acid, 3-methyl-, ethyl est	87	847	853	-6	Ester	18024932.23	17710191.17	16734851.82	29554291.43	29297437.10	29169505.00	6955073.06	6724069.09	7746794.78
Benzophenone	105	1633	1635	-2	Ketone	559771.79	397072.47	394311.52	430460.95	413695.35	335697.96	8495636.70	8466046.68	8264260.07
Isophorone	82	1119	1123	-4	Ketone	8515999.39	8693269.87	8883504.93	324433.92	327277.91	365723.98	645099.19	579562.71	675847.41
Propanoic acid, 2-methyl-, ethyl e	89	752	756	-4	Ester	7700251.45	7752174.96	7508571.57	10200125.88	10312846.14	10049545.23	4172887.82	4021670.91	4109767.58
Butanoic acid, ethyl ester	117	793	802	-9	Ester	4723084.27	4750244.49	4755851.34	3432975.66	3409922.76	3376831.47	1657913.08	1604631.52	1629630.43
Butanal, 3-methyl-	58	666	652	14	Aldehyde	78154972.76	79175992.33	82455206.84	85741493.15	84379708.01	86135951.87	201343677.74	196512026.91	195569506.15
2-Hexanone	100	781	790	-9	Keton	1032492.51	1046233.39	1084848.08	2067415.61	2010943.88	2071105.14	9502913.46	9112603.77	9653101.29
Butanoic acid, 2-methyl-, ethyl est	102	844	849	-5	Ester	34270995.83	33174474.03	32557665.10	48020916.28	46351808.03	45585115.32	9513066.67	8876578.45	9314058.18
Butanoic acid, ethyl ester	37	793	802	-9	Ester	18911870.95	19169643.53	19420490.21	21801673.81	21269492.28	21403737.02	7507655.37	7164460.93	7745056.70
Pentanoic acid, ethyl ester	84	894	900	-6	Ester	28532998.63	28049792.95	27219832.72	25887893.69	24831289.87	24587940.67	11793746.65	12515217.72	11545490.09
1-Hexanol	85	862	868	-6	Alcohol	8717237.45	8826757.14	9268297.92	8571540.49	8454105.32	8538256.62	12176142.12	11777550.65	12295630.02
1-Butanol	57	672	659	13	Alcohol	57685310.88	58767018.58	58436506.90	57936951.76	62350532.70	65287880.97	137399062.90	136588226.07	136851932.55
Octane	56	791	800	-9	Alkane	19004223.57	19836794.32	20610726.80	14221017.56	14431590.05	14166006.39	72963527.66	72705133.42	72522714.28
Butanoic acid, 3-methyl-	69	831	850	-19	Acid	3388296.95	3358855.61	3650512.28	2785483.28	2898842.91	2634628.90	4921741.11	4938462.79	4748656.84
5-Methylhexanoic acid	129	1067	1057	10	Acid	632182.16	576387.12	532194.47	733690.15	618188.50	630818.40	2305297.74	2158231.17	2119490.10

Figure 3: Snapshot of the feature table of the Baijiu ChromaTOF Tile processing.

The profile and trend of one of the meaningful features are shown in detail in Figure 4. The mass spectral comparison with the NIST MS library provides information for the compounds' identification suggesting 2-pentanone. The similarity match was 952/1000 with a RI difference of 4 RI units. This analyte was mainly observed in sample class Aa4, Aa1, and Aa2, though it was present in lower abundance in the other samples and least detected in Cab1 and Cab2. The compound 2-pentanone has fruity-type odor descriptors and it is associated with fruit and alcoholic flavors; it has a sweet banana-like character with fermented, woody nuances.

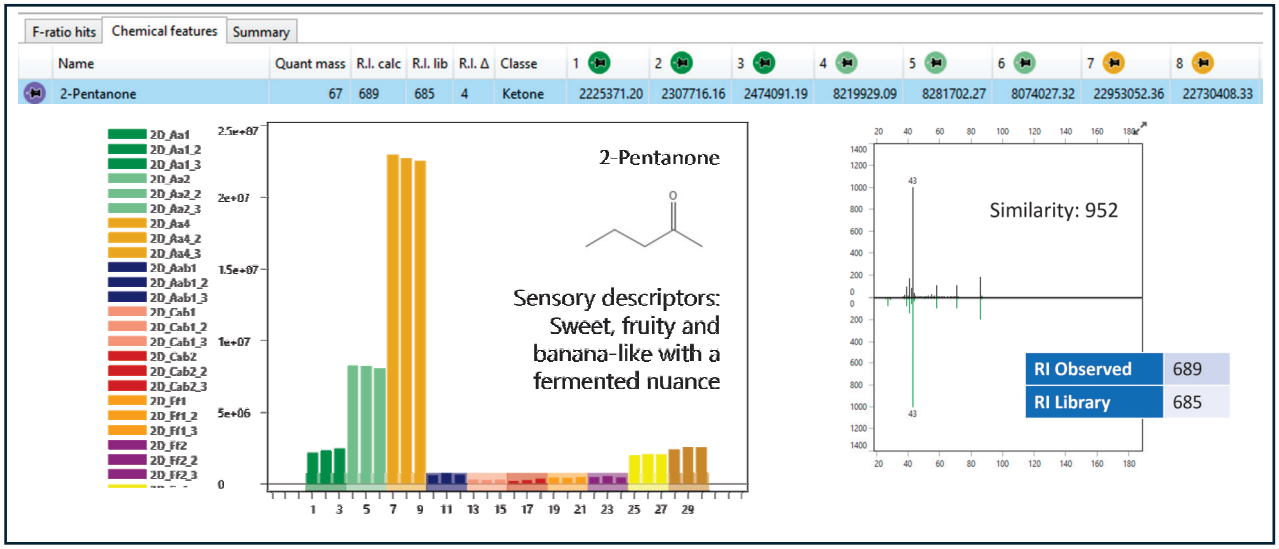

Figure 4: Relative trend of 2-pentanone and its identification assessment criteria.

Conclusion

In this Application Note, the use of HS-SPME injection and GCxGC-TOFMS analysis combined with statistical data processing to analyze the VOCs from an alcoholic beverage is reported. The easy and user-friendly approach to the differentiative analysis was demonstrated on a data set of 10 Baijiu samples. Baijius from different geographical origin were classified into four categories based on their aroma profile. Understanding trends 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.

The non-targeted approach for multiple GCxGC data demonstrates a straight-forward and effective way to explore and interpret results obtained from such a rich data set. Group-type differentiating compounds such as e.g., 2-pentanone were easily highlighted by the Fisher-ratio ranking within *ChromaTOF* Tile. Compounds of interest were identified based on comparison of mass spectral information with the NIST MS library and retention index calculations. 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. Thus, the capability of *ChromaTOF* Tile software to facilitate a fast class differentiation in a non-targeted approach for multiple GCxGC-TOFMS samples was demonstrated.

References

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

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