APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 276

Comparison of Extraction, GC Separation, and Mass Spectral Detection Techniques for the Determination of 2-Methylisoborneol and Geosmin in Finished Whiskey

Nicole C. Kfoury and Megan C. Harper-Kerr

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA

Keywords

2-Methylisoborneol, Geosmin, Whiskey, Selectable ^{1}D/^{2}D-Gas Chromatography-Mass Spectrometry (^{1}D/^{2}D-GC-MS)

Abstract

Off odors in finished whiskey, such as 2-methylisoborneol (MIB) and geosmin, which impart a musty/earthy odor, can significantly impact its sensory quality, even at trace levels. Various extraction techniques paired with gas chromatography-mass spectrometry (GC-MS) can provide a highly sensitive analysis. However, insufficient GC resolution prevents reliable determination of MIB and geosmin because the target ions are not unique to these compounds. A Selectable ^{1}D/^{2}D-Gas Chromatography-Mass Spectrometry (^{1}D/^{2}D-GC-MS) instrument provides an effective way of improving the separation and reliability of the analysis. The enhanced separation removes interferences from the complex whiskey matrix that prevent accurate quantitation in a single-dimensional separation.

Introduction

2-Methylisoborneol (MIB) and geosmin are naturally occurring metabolites that bacteria and fungi produce. These compounds have odors described as musty, earthy, dirt, potting soil, beets, etc. They have very low odor thresholds and can be smelled at 1-10 or 5-10 ppt in water for geosmin and MIB, respectively. MIB and geosmin are most often found in water sources, including drinking water, and are difficult to remove by conventional water treatment methods. They can also be found in grains if contaminated water is used in irrigation, processing, and storage or if improper storage conditions lead to the growth of mold or bacteria. As a result, products such as whiskey that utilize contaminated water and/or grains may result in musty/earthy off odors in the finished product.

Targeting these low-concentration analytes in a complex mixture such as whiskey requires an instrument with high selectivity and sensitivity. Typically, such targeted analyses would involve sample extraction combined with gas chromatography-mass spectrometry (GC-MSD) in selected ion monitoring (SIM) mode to target the analytes of interest. In addition, a triple quadrupole mass spectrometer (TQ) can be employed to utilize multiple reaction monitoring (MRM). While these single-dimension GC analyses can be highly sensitive and selective, the insufficient GC resolution often prevents reliable determination of the target analytes because the base ions, m/z 95 for MIB and m/z 112 for geosmin, are not unique to these compounds and interferences are commonly present in complex sample matrices. A two-dimensional GC-MS provides an effective way to improve analyte separation and remove interferences.

The Selectable ^{1}D/^{2}D-Gas Chromatography-Mass Spectrometry (^{1}D/^{2}D-GC-MS) or "heart-cutting" GC can resolve components in complex matrices. The system is configured with two low thermal mass (LTM) GC columns with orthogonal column phases and a valveless, software-controlled switching device to easily implement a ^{2}D GC separation. The LTM columns provide rapid heating and cooling for fast GC and independent temperature control for multi-dimensional GC. The fast temperature programming can shorten analysis time while improving analyte resolution through

GERSTEL AppNote 276

heart-cutting increases quantitation accuracy for high-throughput screening of MIB and geosmin.

This study compares the determination of MIB and geosmin in finished whiskey at the ppt level using GC-MSD in SIM mode, GC-TQ in MRM mode, and ^{1}D/^{2}D-GC-MSD in SIM mode. Samples are extracted via two widely used solventless extraction techniques; solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE). The analysis methods are evaluated based on linearity, detection limit, reproducibility, and accuracy of measuring the target analytes.

Experimental

Instrumentation

GERSTEL MPS robotic^{pro} with SPME on Agilent 8890/5977B GC/MSD

GERSTEL MPS robotic^{pro} with SPME on Agilent 8890/7010B GC/TQ

GERSTEL LabWorks Platform with SPME on Agilent 8890/5977C GC/MSD with LTM option as shown in Figure 1.

Figure 1: Selectable ^{1}D/^{2}D-GC-MSD system (optional ODP and DHS shown).

Analysis Conditions SPME

Fiber	DVB/CAR/PDMS, 2 cm
MPS	65 °C incubation/extraction temperature
	3 min incubation time
	30 min extraction time
	750 rpm stirring speed
CIS 4	SPME liner
	splitless
	270 °C isothermal

Analysis Conditions LabWorks Platform Twister®

Twister®	PDMS
TDU 2	splitless
	40 °C; 720 °C/min; 280 °C (3 min)
CIS 4	glass bead-filled liner
	solvent vent (50 mL/min), split 10:1
	-120 °C; 12 °C/sec; 280 °C (3 min)

Analysis Conditions Agilent 8890 GC

Column	30 m HP-5MS (Agilent)
	d_i = 0.25 mm, d_f = 0.25 µm
Pneumatics	He, P_i = 10.42 psi
Oven	60 °C (2 min); 8 °C/min; 280 °C

Analysis Conditions Agilent 8890 GC with LTM option

Column 1	LTM Format, 30 m DB-WAX (Agilent)
	d_i = 0.25 mm, d_f = 0.25 µm
	40 °C (1 min); 15 °C/min; 240 °C (0 min)
Column 2	LTM Format, 30 m DB-5MS UI (Agilent)
	d_i = 0.25 mm, d_f = 0.25 µm
	40 °C (13.8 min); 15 °C/min; 280 °C (1 min)
Pneumatics	He, P_i = 335.17 kPa
	constant pressure (^{1}D)
	ramped pressure with backflush (^{2}D)
Oven	250 °C, isothermal

Analysis Conditions 5977B and 5977C MSD

Scan	40 – 300 m/z
SIM	95 and 108 m/z (MIB)
	195 and 197 m/z (TCA)
	112 and 125 m/z (Geosmin)

Analysis Conditions 7010B TQ

MRM	95 → 67 and 95 → 55 m/z (MIB)
	210 → 95 and 212 →197 m/z (TCA)
	112 → 97 and 112 → 83 m/z (Geosmin)

GERSTEL AppNote 276

Standard/Sample Preparation

A 100 µg/mL drinking water odor standard containing MIB and geosmin in methanol was purchased from Restek (Part # 30608). Neat 2,4,6-trichloroanisole (TCA) was purchased from Sigma Aldrich (Part # 235393) and diluted in methanol. Five whiskeys were purchased at a local store.

Standards Preparation

A five-point matrix-matched calibration curve was prepared for each analyte from 5-100 ppt. Approximately 2.4 g of dried NaCl was added to a 20 mL screw-capped vial. A 9.9 mL aliquot of water and 0.1 mL aliquot of ethanol were added to the vial to make a 1% ethanol solution. The appropriate MIB/Geosmin standard volume was spiked for each calibration level. The internal standard, TCA, was spiked at 100 ppt in each calibration standard. Each calibration point was prepared in duplicate.

Sample Preparation

All whiskey samples were diluted to 1% ethanol for SPME extractions. Approximately 2.4 g of dried NaCl was added to a 20 mL screw-capped vial. A 9.8-9.75 mL aliquot of water and 0.2-0.25 mL aliquot of whiskey were added to the vial. The internal standard, TCA, was spiked at 100 ppt in each sample. A non-coated stir bar was placed in the vial.

Whiskeys 1 and 2 were diluted to 1% or 20% ethanol for Twister extractions. The appropriate water and whiskey volumes were added to a 10 mL screw-capped vial. The internal standard, TCA, was spiked at 100 ppt in each sample. A PDMS Twister stir bar was immersed in each sample. The samples were stirred at 1100 rpm for 1 hour at room temperature. After extraction, the Twister stir bar was removed, rinsed with water, and blotted dry before placing it in an empty TD tube. The TD tube was sealed with a transport adapter and placed in a 40-position tray on the MPS LabWorks Platform system for automated analysis.

Standard/Sample Introduction

The standards and samples in 20 mL screw-capped vials were placed on a VT15 tray on the MPS for SPME extraction. The samples were incubated at 65 °C for 3 minutes with a stir speed of 750 rpm. Then, the sample headspace was extracted for 30 minutes. The analytes were trapped on a CAR/DVB/PDMS 2 cm fiber. The SPME fiber was desorbed at 270 °C in the CIS 4 inlet in splitless mode for 3 minutes.

The Twister stir bars were desorbed in splitless mode under a 50 mL/min helium flow at 280 °C for 3 minutes. Analytes were cold trapped in the CIS 4 inlet at -120 °C on a glass bead-filled liner. When desorption was complete, analytes were transferred to the column in split (10:1) mode by heating the inlet rapidly to 280 °C.

Results and Discussion

The sample extraction parameters were optimized before the instrument comparison study using SPME extraction. A 1 ppb MIB and geosmin standard was extracted with varying ethanol concentrations to determine the effect of ethanol content on SPME extraction efficiency. Table 1 shows the percent difference in peak signal between the standards extracted with no ethanol and 1-5% ethanol. While geosmin is only slightly affected by the increased ethanol content, the MIB signal is reduced by almost half with 5% ethanol present. As a result, 1% ethanol content was chosen for all remaining SPME extractions.

Table 1: Percent difference in peak signal between standards extracted using SPME with varying ethanol concentrations.

Ethanol %	MIB	Geosmin
0	100	100
1	88	98
3	70	95
5	58	92

For each instrument, matrix-matched calibration curves with 1% ethanol in water were generated in the working range from 5 – 100 ppt. Salt was added to the sample before extraction to increase the target analytes' concentration in the headspace. Linearity was excellent, with correlation coefficients (R^2) greater than 0.99 for both analytes, as shown in Table 2. The detection limit (DL) for each analyte was below 5 ppt, also shown in Table 2. As expected, the $^1D/^2D$ instrument has marginally higher detection limits due to the extra flow inputs at the Dean's switch and purged splitter, which causes a dilution effect within the system.

Table 2: Linearity and detection limit (ppt) for MIB and geosmin.

	MSD		TQ		$^1D/^2D$	
	R^2	DL	R^2	DL	R^2	DL
MIB	0.997	2.15	0.997	1.44	0.992	3.94
Geosmin	0.995	2.16	0.997	1.86	0.995	4.05

To assess reproducibility, one standard was analyzed in triplicate on each instrument. The percent relative standard deviation (%RSD) for both analytes on each instrument is less than 5%, as shown in Table 3.

Table 3: Reproducibility (%RSD) of MIB and geosmin.

	MSD	TQ	$^1D/^2D$
MIB	4.3	2.3	3.7
Geosmin	2.6	2.4	2.3

When calibration standards were analyzed, the three instruments performed similarly regarding linearity, detection limit, and reproducibility. However, the results were quite different when assessing the quantitation of MIB and geosmin in the five whiskey samples. Tables 4 and 5 show the concentration (ppt) of MIB and geosmin in the whiskey samples measured on each instrument.

The concentration values for the GC-MSD running in SIM mode are very high, reaching the low ppb range for MIB and the high ppt range for geosmin in specific samples. These concentration values, if accurate, are expected to result in consumer dissatisfaction. SIM mode can be very useful in enhancing detection limits and selectivity of analysis by focusing on specific ions rather than scanning over a wide range of m/z values. However, the resulting concentration values may be inflated if the target ions are not free from interference. For MIB and geosmin, the base ions m/z 95 and 112 are not unique to these analytes. In addition, the remaining ions in each spectrum are relatively low in abundance, which can negatively impact signal-to-noise ratio, detection limit, and accuracy, resulting in less reliable quantitation. Due to the complex matrix of whiskey, matrix components coelute with the target analytes, causing ion interferences, which leads to exaggerated concentration values.

Table 4: Concentration (ppt) of MIB in whiskey samples measured on each instrument.

	MSD	TQ	$^1D/^2D$
Whiskey 1	341	362	n.d.
Whiskey 2	274	n.d.	n.d.
Whiskey 3	272	367	n.d.
Whiskey 4	1,364	516	n.d.
Whiskey 5	1,310	417	n.d.

Note: n.d. = not detected

In MRM mode, precursor-to-product ion transitions, which are often unique to a target compound, are monitored. For MIB and geosmin, the base ions m/z 95 and 112 are not unique to these analytes but must be used as the precursor ions due to the low abundance of the remaining ions in each spectrum. The product ions should be abundant fragments, consistently produced, and, if possible, unique to the target compound. The ions m/z 55 and 67 are used for MIB, and m/z 97 and 83 are chosen for geosmin. These product ions are low in abundance and are not highly unique, especially for MIB, which once again results in interferences from coeluting compounds in the complex matrix and inflated concentration values. The use of MRM reduces interferences compared to MSD SIM mode, and the concentrations from the GC-TQ analysis are significantly lower than the GC-MSD. However, some values are still in the 100s of ppt range, which could cause consumer complaints.

Table 5: Concentration (ppt) of geosmin in whiskey samples measured on each instrument.

	MSD	TQ	$^1D/^2D$
Whiskey 1	523	119	273
Whiskey 2	579	202	120
Whiskey 3	850	232	87
Whiskey 4	714	165	91
Whiskey 5	621	187	81

The $^1D/^2D$-GC-MSD system results show that MIB is undetected in all the samples, and the geosmin concentration is around 100 ppt for all but one sample. At these concentrations, there would likely be no cause for complaints, and it is clear that the ^1D-GC analysis of these compounds in finished whiskey suffers from ion interferences from coeluting compounds even with the GC-TQ in MRM mode. The $^1D/^2D$-GC-MSD overcomes the interferences by using two columns with orthogonal stationary phases to separate the analytes of interest from the complex matrix, resulting in more accurate concentration values.

To be confident that the considerable dilution of the samples to 1% ethanol didn't dilute out the MIB, samples were extracted using Twister stir bar sorptive extraction, a higher-capacity extraction technique than SPME. Once again, the ethanol content of the samples was optimized. Table 6 shows the percent difference in peak signal between the standards extracted with no ethanol and 1-20% ethanol. Unlike the SPME extraction, neither MIB nor geosmin were affected by the increased ethanol content. The limited

sorbent phase available on an SPME fiber results in competition effects, which are eliminated with the Twister stir bars. Therefore, the whiskey samples with 40-50% ethanol content only needed to be diluted in half for the Twister extraction.

Table 6: Percent difference in peak signal between standards extracted using Twisters with varying ethanol concentrations.

Ethanol %	MIB	Geosmin
0	100	100
1	103	97
3	96	105
5	96	107
20	97	98

Whiskeys 1 and 2 were extracted with the Twister stir bar at 1 and 20% ethanol. MIB remained undetectable in all the extractions. The relative peak area of geosmin was similar for the SPME and Twister extractions, regardless of the dilution, as shown in Table 7.

Table 7: Relative peak area of geosmin.

	SPME 1%	Twister 1%	Twister 20%
Whiskey 1	1.94	1.99	1.96
Whiskey 2	0.96	0.88	0.90

Conclusion

This study demonstrated the ability of the $^{1}D/^{2}D$-GC-MSD system to eliminate interferences and measure MIB and geosmin concentrations in finished whiskey accurately. Due to the complex whiskey matrix, single-dimensional GC separations, even with SIM and MRM detection modes, suffer from interferences from coeluting compounds, resulting in inaccurate concentration measurements. Extracting complex samples like whiskey with SPME is prone to competition effects, and as a result, samples must be significantly diluted before extraction, causing detection limits to suffer. Twister stir bars can be used without significant dilution or salting out. In addition, the Twister stir bars can increase analyte mass on column for other analytes of interest and significantly decrease detection limits for the analysis.