

Delivering the Right Results

Analysis of Trace Odorants in Drinking Water by Headspace-Solid Phase Microextraction (HS-SPME) with Gas Chromatography-Time of Flight Mass Spectrometry (GC-TOFMS) and Comprehensive Two-Dimensional Gas Chromatography-Time of Flight Mass Spectrometry (GCxGC-TOFMS)

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INTRODUCTION

Odor causing compounds can find their way into drinking water from a number of sources. Odors with a "musty" character are often attributed to chemical byproducts from several classes of microbes. One of the most common compounds causing a "musty" or "earthy" smell is geosmin which is released when microbes such as blue-green algae and actinobacteria die. Geosmin is of particular interest since it has a very low odor threshold. Many people are able to detect the smell of geosmin at concentrations as low as 10 parts per trillion (ppt). Other well known odor causing compounds that originate from natural sources include 2-methylisoborneol and methoxypyrazines. Since many of these odorants have sensory thresholds in the ppt range, it is important to have an analytical method capable of detecting them at trace levels. This poster highlights a modified method 6040D showing an automated Headspace-Solid Phase Microextraction (HS-SPME) sampling approach. This sampling method coupled with GC-TOFMS and GCxGC-TOFMS provides both sensitivity for targeted odorants as well as the selectivity to detect and identify non-targeted odorant compounds.

Odorants can also originate from packaging materials such as plastic bottles. These compounds are often oxidation products such as aldehydes. Odorants which originate from packaging materials will not be discussed in this poster, but may be part of future studies, since bottled water is an \$8 billion industry in the US alone.

SAMPLES

Water odorant standards containing geosmin, 2-methylisoborneol (MIB), 2-isopropyl-3-methoxypyrazine (IPMP), and 2-isobutyl-3methoxypyrazine (IBMP) at concentrations ranging from 1.0 to 100 ppt were prepared using water odorant standards purchased from Supelco (Bellefonte, PA). An internal standard (2,4,6-trichloroanisole) was used in each water standard at a concentration of 10 ppt. A 10-mL aliquot of each water standard was placed in respective 20 mL headspace autosampler vials which contained 2.5 grams of NaCl. This "salting out" procedure was used to force the analytes of interest into the headspace where they are collected by the SPME fiber. These standards were analyzed by Headspace-Solid Phase Microextraction-Gas Chromatography-Time of Flight Mass Spectrometry (HS-SPME-GC-TOFMS) and Headspace-Solid Phase Microextraction-Comprehensive Two-Dimensional Gas Chromatography-Time of Flight Mass Spectrometry (HS-SPME-GCxGC-TOFMS) using the conditions detailed in the Experimental section.

EXPERIMENTAL

SPME Extraction and Sample Introduction

Gerstel MPS2 Autosampler w/SPME Option (Gerstel US, Linthicum, MD) SPME Fiber: 2 cm, Divinylbenzene / Carboxen / Polydimethylsiloxane (Supelco, Bellefonte, PA)

Incubation Temperature: 65°C

Incubation Time: 30 min, with SPME fiber exposure

Agitator Speed: 100 rpm (10 s duration, every 60s) Desorb Time: 3 min @ 260°C

One-Dimensional GC-TOFMS (LECO TruTOF)

GC: Agilent 6890 Gas Chromatograph

Injection: SPME, Splitless (60 s Purge Time) @ 260°C Carrier Gas: He @ 1.5 mL/min

Column: Rxi-5ms; 20 m x 0.18 mm x 0.18 m (Restek Corporation, Bellefonte, PA) GC Oven: 60°C (2 min hold), programmed @ 30°C/min to 250°C (10 min hold)

MS Transfer Line: 280°C

MS: LECO TruTOF® HT

Ion Source Temperature: 300°C

Spectral Acquisition Rate: 20 spectra/s Acquired Mass Range: 45-300 m/z

Comprehensive Two-Dimensional GCxGC-TOFMS (LECO Pegasus 4D)

GC: Agilent 6890 w/LECO thermal modulator and secondary oven

Injection: SPME, Splitless (60 s Purge Time) @ 260°C

Carrier: Helium at 1.5 ml/min, corrected constant flow Primary Column: Rxi-5 Sil MS, 30 m x 0.25 mm x 0.25 μ m

Primary Oven: 60°C hold 2 min, 10°C/min to 260°C, hold 20 min Secondary Column: Rtx-200, 1.5 m x 0.18 mm x 0.2 μ m (Restek Corporation, Bellefonte, PA)

Secondary Oven: +5°C offset from primary oven

Modulator Offset: 15°C (relative to the secondary oven)

Modulation Period: 4 s Transfer Line Temp: 280°C

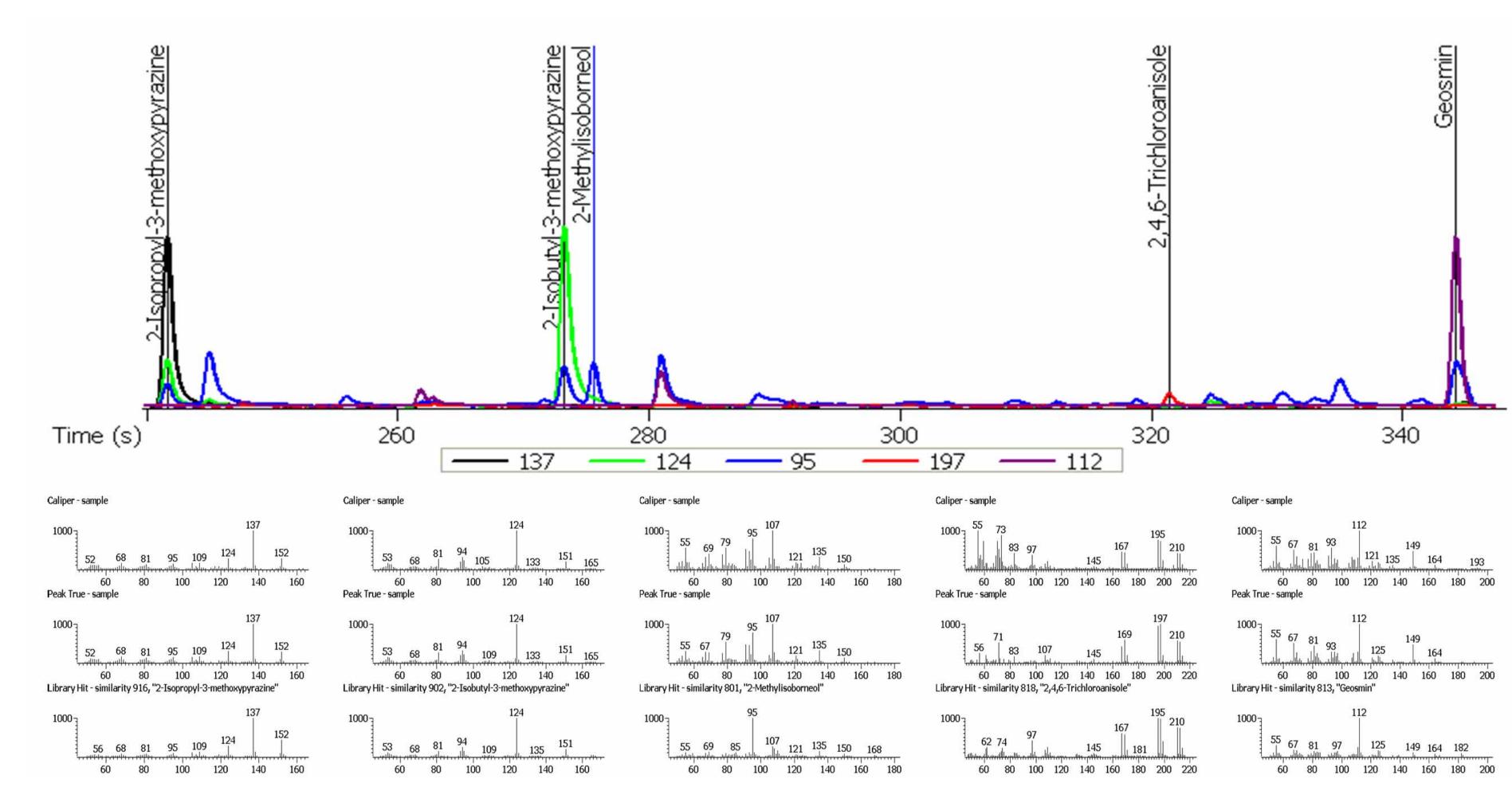
MS: LECO Pegasus® 4D Ion Source Temperature: 225°C

Spectral Acquisition Rate: 200 spectra/s Acquired Mass Range: 45-300 m/z

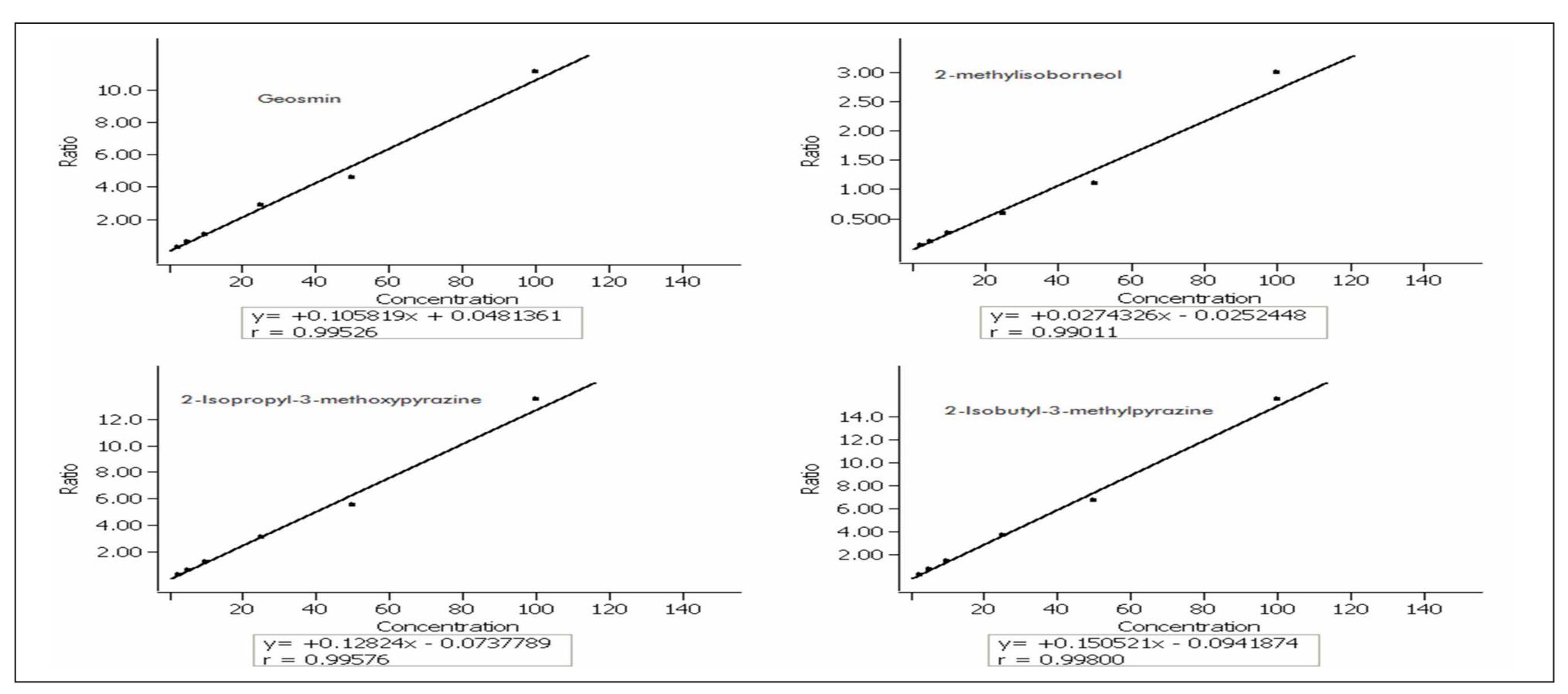
Instrument Control and Data Review:

ChromaTOF®

RESULTS AND DISCUSSION (GC-TOFMS)



The figure above is an extracted ion chromatogram (EIC) of the quant masses for IPMP(m/z 137), IBMP(m/z 124), MIB(m/z 95), 2,4,6-TCA(m/z 197), and geosmin(m/z 112) showing the chromatographic separation for these odorant compounds at 100 ppt. The Caliper (raw), Peak True (deconvoluted), and Library Hit spectra for each compound are shown below the EIC

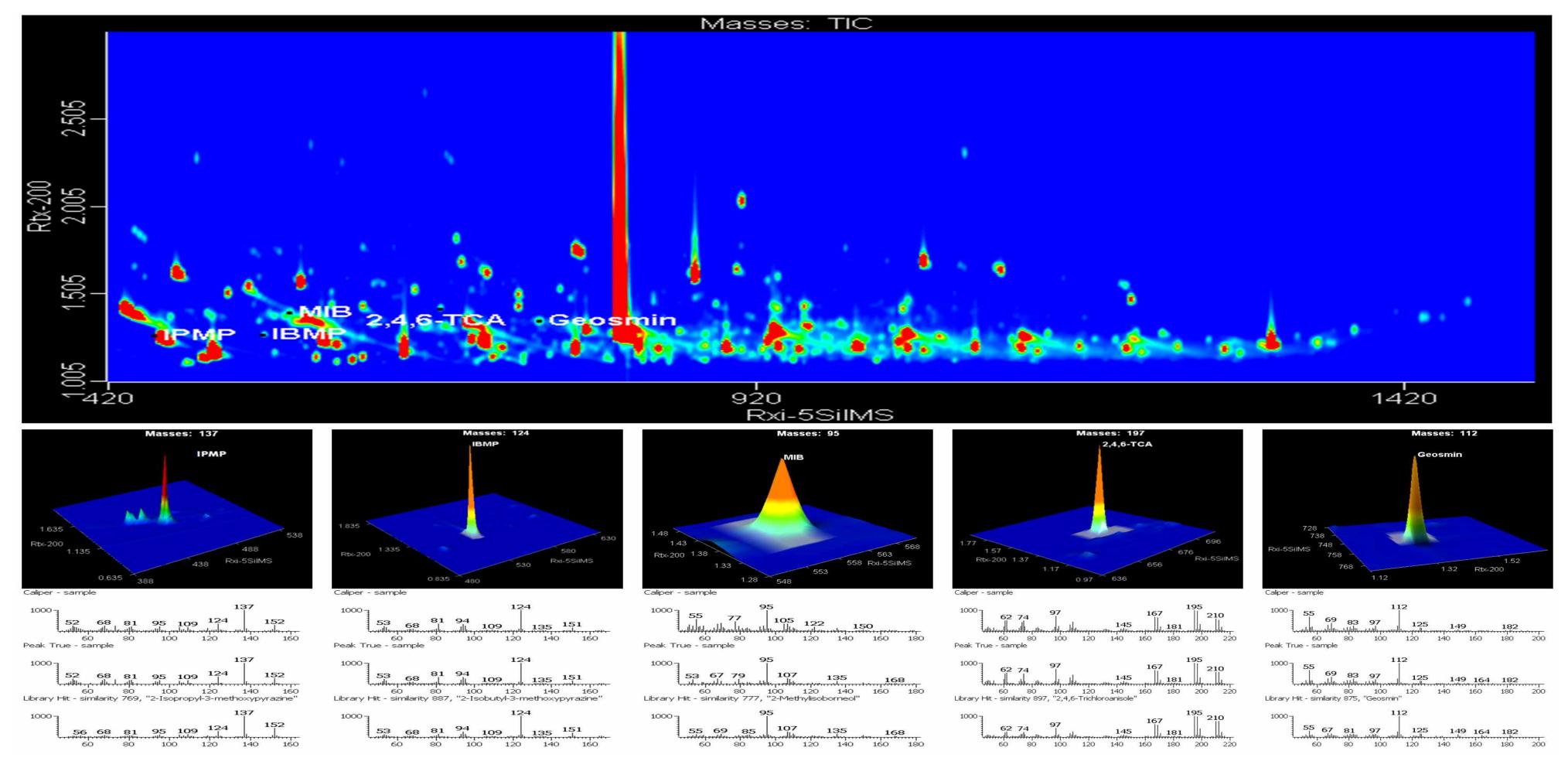


The figure above shows the calibration curves for each of the odorant compounds. This HS-SPME-GC-TOFMS method shows exceptional linearity across the calibration range shown (2.5 - 100 ppt) for all four compounds.

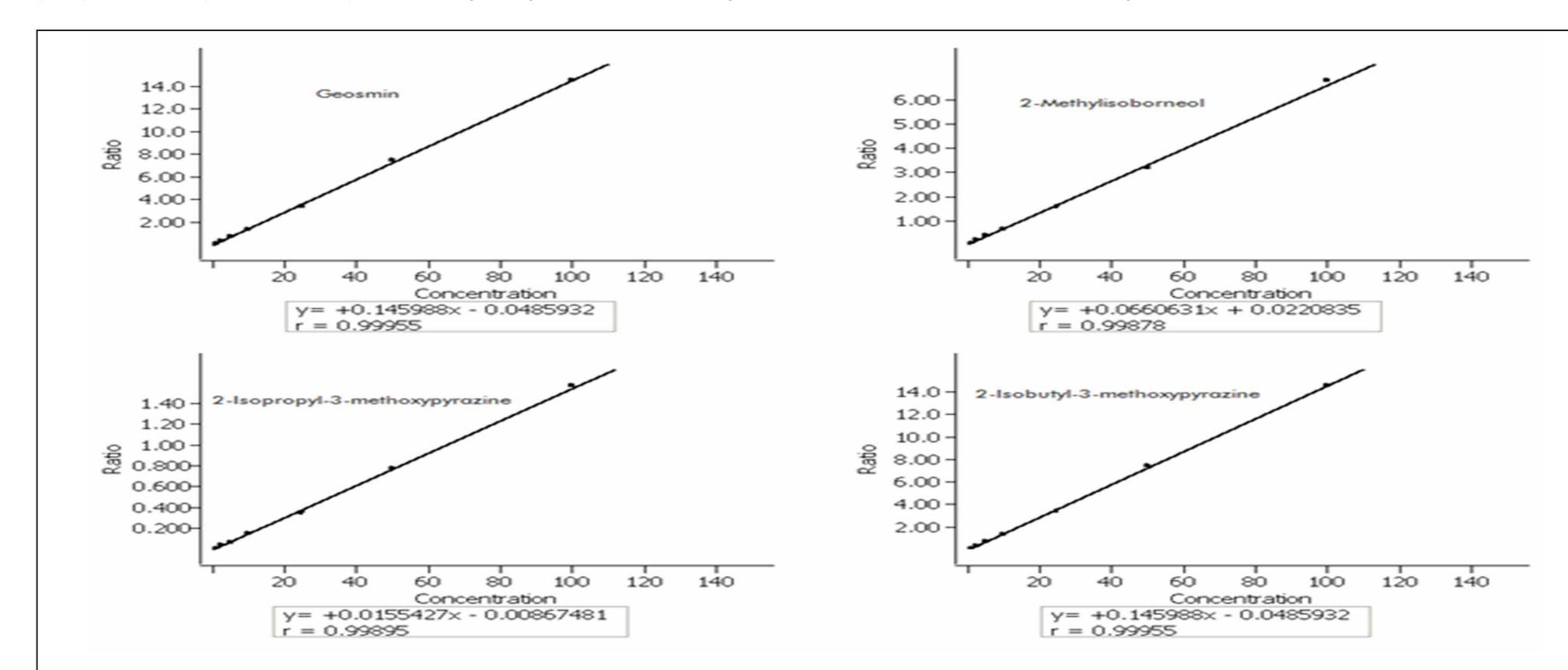
Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N	Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N
Geosmin @ 2.5 ppt	326284	2.5	2.3694	5.225	328.25	2-Methylisoborneol @ 2.5 ppt	56432	2.5	2.8045	12.178	50.706
Geosmin @ 5.0 ppt	760248	5	5.5089	10.178	857.14	2-Methylisoborneol @ 5.0 ppt	137889	5	5.0927	1.854	116.94
Geosmin @ 10.0 ppt	941622	10	9.6344	3.6564	1118.5	2-Methylisoborneol @ 10.0 ppt	227849	10	10.338	3.375	210.08
Geosmin @ 25.0 ppt	2642013	25	26.85	7.4019	3179.9	2-Methylisoborneol @ 25.0 ppt	536386	25	22.304	10.784	519.94
Geosmin @ 50.0 ppt	5078272	50	43.164	13.671	5650.6	2-Methylisoborneol @ 50.0 ppt	1222192	50	41.415	17.17	1227.9
Geosmin @ 100.0 ppt	8977964	100	104.97	4.9725	10124	2-Methylisoborneol @ 100.0 ppt	2420154	100	110.55	10.546	2056.9
Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N	Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N
2-Isopropyl-3-methoxypyrazine @ 2.5 ppt	292587	2.5	2.6651	6.6049	288.52	2-Isobutyl-3-methoxypyrazine @ 2.5 ppt	304347	2.5	2.4778	0.88998	331.52
2-Isopropyl-3-methoxypyrazine @ 5.0 ppt	673515	5	4.935	1.2999	682.75	2-Isobutyl-3-methoxypyrazine @ 5.0 ppt	819541	5	5.1454	2.9076	823.12
2-Isopropyl-3-methoxypyrazine @ 10.0 ppt	1096350	10	10.269	2.6862	1138.4	2-Isobutyl-3-methoxypyrazine @ 10.0 ppt	1271139	10	10.201	2.0077	1264.2
2-Isopropyl-3-methoxypyrazine @ 25.0 ppt	2814418	25	24.577	1.692	2855	2-Isobutyl-3-methoxypyrazine @ 25.0 ppt	3394527	25	25.289	1.1575	3333.7
2-Isopropyl-3-methoxypyrazine @ 50.0 ppt	6076919	50	43.646	12.707	6205.7	2-Isobutyl-3-methoxypyrazine @ 50.0 ppt	7419876	50	45.431	9.139	7264.3
	10922025	100	106.41	6.4078	10890	2-Isobutyl-3-methoxypyrazine @ 100.0 ppt	12516652	100	103.96	3.9562	11560

The tables above show the tabular data for each of the water odor compounds by GC-TOFMS. Notice how the results shown in the "Calculated Concentration" columns correlate well with the "Certified Concentration" columns. These show the accuracy of the calculated concentration of each individual standard based on the slope of the curve. The "% Difference Concentration" columns show that the error between certified vs. calculated concentration is < 10% for most standards

RESULTS AND DISCUSSION (GCxGC-TOFMS)



The figure above shows a total ion chromatogram (TIC) of a GCxGC-TOFMS contour plot which represents an x-axis separation on an Rxi-5SilMS column and a y-axis separation on an Rtx-200 column for odorant components at 25ppt. Directly underneath the contour plot are extracted ion 3D surface plots of the quant masses for the odorant compounds IPMP(m/z 137), IBMP(m/z 124), MIB(m/z 95), 2,4,6-TCA(m/z 197), and geosmin(m/z 112). The Caliper (raw), Peak True (deconvoluted), and Library Hit spectra for each compound are shown below the 3D surface plots.



The figure above shows the calibration curves for each of the odorant compounds. This HS-SPME-GCxGC-TOFMS method shows exceptional linearity across the calibration range shown (1 – 100 ppt) for all four compounds. Notice: this technique provided the ability to quantitate down to 1 ppt for all four analytes. This is due to the cryo-focusing effects of thermally modulated GCxGC which improves analyte detectability. See diagram shown in observations

Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N	Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N
Geosmin @ 1.0ppt	90839	1	0.99954	0.045715	84.652	2-Methylisoborneol @ 1.0 ppt	32915	1	0.9062	9.3804	77.34
Geosmin @ 2.5 ppt	190832	2.5	2.4907	0.37066	1142.8	2-Methylisoborneol @ 2.5 ppt	80657	2.5	2.6449	5.7968	186.62
Geosmin @ 5.0 ppt	351466	5	5.0222	0.44423	2200.6	2-Methylisoborneol @ 5.0 ppt	159657	5	5.6633	13.265	345.81
Geosmin @ 10.0 ppt	671632	10	9.9229	0.77094	4209.2	2-Methylisoborneol @ 10.0 ppt	270160	10	9.7688	2.3117	692.82
Geosmin @ 25.0 ppt	1683806	25	24.642	1.433	11333	2-Methylisoborneol @ 25.0 ppt	656506	25	23.41	6.3599	1760.3
Geosmin @ 50.0 ppt	3250773	50	51.753	3.5064	22611	2-Methylisoborneol @ 50.0 ppt	1236059	50	47.883	4.2338	3519.2
Geosmin @ 100.0 ppt	6755022	100	98.67	1.3304	43498	2-Methylisoborneol @ 100.0 ppt	2904247	100	103.22	3.2236	7459.9
Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N	Standard	Area	Cert. Conc.	Calc. Conc.	% Diff. Conc.	Quant S/N
2-Isopropyl-3-methoxypyrazine @ 1.0ppt	2611.8	1	0.97651	2.3492	8.9468	2-Isobutyl-3-methoxypyrazine@ 1.0ppt	45437	1	1.1078	10.776	447.36
2-Isopropyl-3-methoxypyrazine @ 2.5 ppt	14588	2.5	2.8484	13.937	36.643	2-Isobutyl-3-methoxypyrazine @ 2.5 ppt	117124	2.5	2.2906	8.3778	1089.5
2-Isopropyl-3-methoxypyrazine @ 5.0 ppt	26053	5	4.718	5.6408	76.047	2-Isobutyl-3-methoxypyrazine @ 5.0 ppt	284632	5	5.1714	3.4274	2311.8
2-Isopropyl-3-methoxypyrazine @ 10.0 ppt	60243	10	10.134	1.339	144.17	2-Isobutyl-3-methoxypyrazine @ 10.0 ppt	550288	10	9.6454	3.5464	4477.3
2-Isopropyl-3-methoxypyrazine @ 25.0 ppt	143011	25	22.543	9.8279	392.96	2-Isobutyl-3-methoxypyrazine @ 25.0 ppt	1428016	25	23.705	5.1804	11948
	299772	50	50.262	0.52373	776.67	2-Isobutyl-3-methoxypyrazine @ 50.0 ppt	2888418	50	51.321	2.6415	24953
2-Isopropyl-3-methoxypyrazine @ 50.0 ppt	233,12	1-0.0									

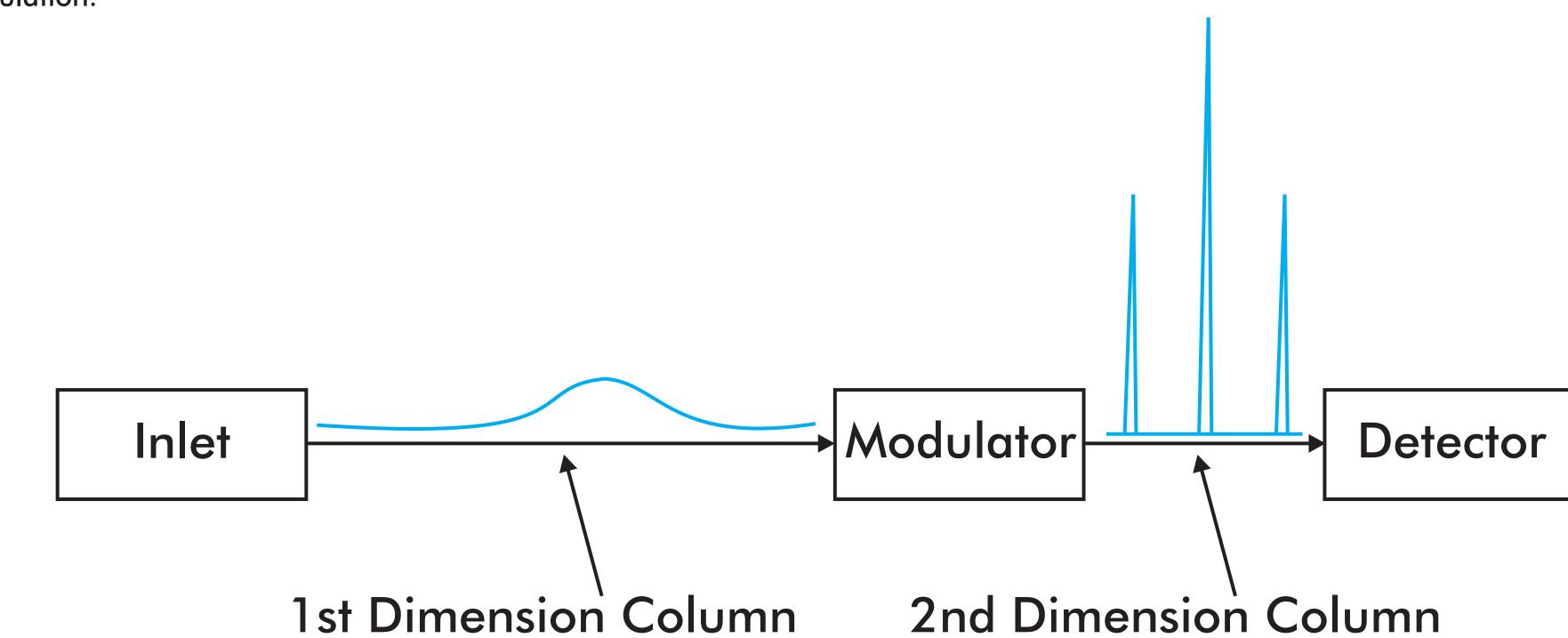
The tables above show the tabular data for each of the water odor compounds by GCxGC-TOFMS. Notice how the results shown in the "Calculated" Concentration" columns correlate well with the "Certified Concentration" columns. These show the accuracy of the calculated concentration of each individual standard based on the slope of the curve. The "% Difference Concentration" columns show that the error between certified vs. calculated concentration is < 10% for most standards.

OBSERVATIONS

-GC-TOFMS and HS-SPME-GCxGC-TOFMS exhibited the ability to detect odorant compounds in water at low ppt levels. A major benefit of TOFMS for this application is the ability to reach these levels of detection while acquiring across a mass range of 45-300 m/z without sacrificing speed or sensitivity. This allows the analyst to confirm analyte identity using the deconvoluted full range mass spectrum, and to detect non-targeted compounds that may also be contributing to the aroma profile of the water sample. Quadrupole MS systems operating in selected ion monitoring (SIM) mode could not accomplish either of these functions.

This HS-SPME-GC-TOFMS method was able to demonstrate linearity across a calibration range of 2.5 – 100 ppt, while the HS-SPME-GCxGC-TOFMS method was able to extend this detection even lower, demonstrating linearity from 1-100 ppt.

GCxGC-TOFMS provides additional chromatographic resolution and increased detectability through the cryo-focusing effects of thermal



The image above is a simplified diagram showing a visual representation of the signal-to-noise enhancement provided by the cryo-focusing aspect of thermal modulation. This focusing effect increases the s/n of a chromatographic peak through conservation of volume allowing an increase in analyte

CONCLUSIONS

The experiments described in this presentation demonstrate the use of the LECO TruTOF (GC-TOFMS) and Pegasus 4D (GCxGC-TOFMS) for the analysis of odorant components in drinking water per a variation of the American Water Works Association (AWWA) method 6040D. This method varied from 6040D in that it required less water than the original method. Method 6040D requires 45 mL while this method requires 10 mL. The ability to use 10 mL of water instead of 45 mL allows for easier automation through the use of the Gerstel MPS2 autosampler which utilized 20 mL headspace vials for these analyses.

REFERENCES

. Method 6040D, Developed by the American Water Works Association (AWWA)

