Application Note: 52242

Identification and Quantification of Impurities in Wines by GC/MS

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Key Words

- ISQ Single Quadrupole GC-MS
- TRACE GC Ultra
- Food and Beverage
- SPME
- Wine

Introduction

While wine makers have historically used gas chromatography and mass spectrometry (GC/MS) to detect pesticides, they now more commonly use the technique to supplement quality control checks of wine taste. Without GC/MS, wine makers must rely on expert evaluation by oenologists to determine wine quality. By identifying maturation tracers and molecules commonly responsible for taste defects, GC/MS augments expert opinion with objective and quantitative information. When using a SPME extraction method, GC/MS has the additional advantages of requiring very small sample sizes, a minimum of sample preparation, and rapid analysis of target molecules.

Several types of molecules, while not dangerous to humans, affect wine taste and quality, such as volatile phenol compounds derived from *Brettanomyces* yeast metabolism.[1,2] Haloanisoles such as 2,4,6-tricholoranisole that result from cork fungal infections also affect wine taste.[3,4] Methoxypyrazines such as 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) are maturation markers, and detecting their levels can help determine ideal grape harvest time.[5] An automated technique with repeatable results for detecting these compounds is highly desirable, and GC/MS can provide such a method.

Extracted wine samples were analyzed by a sequential full-scan/SIM acquisition on a GC-MS system consisting of a Thermo Scientific ISQ single-quadrupole mass spectrometer and a Thermo Scientific TRACE GC Ultra gas chromatograph. The results were compared to the sensitivity limits of human tasters. This method allows wine makers to obtain precise measurements on the organoleptic parameters that determine wine purity on site rather than having to send samples for expensive, external analysis. In this report, we present the design and results of this study, including the experimental method used to detect impurities and the concentration ranges that compare GC/MS with human detection.

Figure 1: ISQ Single Quadrupole GC-MS system

Methods

For this experiment, several targeted molecule types that affect wine quality were analyzed using an ISQ™ Single Quadrupole GC-MS system (Figure 1). Table 1 contains a brief description of the effects on wine quality of the four target molecule types, and examples of how GC/MS analysis can provide value in quality control.

Molecule Type	Description of Effect on Wine	Benefit of GC/MS Analysis
Volatile Phenols (4-ethylphenol, 4-ethylgaiacol, 4-vinylphenol, 4-vinylgaiacol)	Volatile phenols are produced in various steps of *Brettanomyces* yeast metabolism. The two produced in the final step – 4-ethylphenol and 4-ethylgaiacol – give the wine an "animal" taste and depreciates its quality.	GC/MS can detect 4-ethylphenol and 4-ethylgaiacol in lower concentration than human tasters. GC/MS can also detect the presence of 4-vinylphenol and 4-vinylgaiacol, intermediaries in *Brettanomyces* yeast metabolism and allow wine makers to discard contaminated batches.
Geosmine	This fragrant compound derived from moldy grapes interferes with a wine's taste.	Detecting geosmine in wine alerts makers to the presence of mold in their grapes and allows them to locate and treat a contaminated plot of land.
Haloanisoles (TBA, TCA, TeBA, PCA)	These compounds come from halophenols, compounds used to prevent wood degradation in vines. They give wine a moldy odor.	Assays provide information of an organoleptic default in wine production and help identify contamination sources.
Methoxypyranzines (IBMP, IPMP)	IBMP and IPMP are maturation markers, and their levels decrease as wine matures. IBMP gives wine a "green pepper" taste; IPMP imparts an earthy flavor.	Determining the levels of IBMP and IPMP in wine affects harvesting decisions.

Table 1: Targeted molecules affecting wine purity

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Sample Preparation

To prepare the samples, a 10 mL sample of wine was saturated with NaCl. The sample was placed in a vial and extracted using SPME. A PDMS/DVB 65 µm StableFlex™ SPME Fiber (SUPELCO-57293U) was used, and the fiber was exposed to the sample for agitation for 30 minutes at 70 °C at three-second intervals.

Instrumental Analysis

The ISQ mass spectrometer used for this analysis was set to perform sequential full scan/SIM acquisitions. The TRACE™ GC Ultra was equipped with a standard split/splitless injector. The split/splitless injector temperature was set to 220 °C, and a splitless injection was used. The ISQ GC-MS parameters are summarized in Table 2. The analytical column used was a Thermo Scientific TraceGOLD TG-5MS 15 m × 0.25 mm i.d. × 0.25 µm film (PN 26098-1300). TCA d5 was used as an internal standard; its SIM ions are 215 and 217.

The results were analyzed using Thermo Scientific QuanLab Forms software. QuanLab™ Forms automatically tests the expected retention times (RT), actual ratio versus range of tolerance, and the coelution of ions. QuanLab Forms is also Directorate-General for Health and Consumer Protection (SANCO) compliant and can be used in the European Union.

Results

The spectra of the sequential SIM scan can be seen in Figure 2. The SIM ions monitored using the ISQ are listed in Table 3. Figures 3 through 7 present the calibration curves of several of the target molecules at various linearity ranges. Calibration ranges were established according to the range of human perception – and to the range of interest for oenologists – as opposed to instrument performance.

For all these target molecules, the GC-MS was able to detect lower concentrations than the limits of human perception.

ISQ	
Source Temp (°C)	200
Detector Gain	1×10^5
Start Time (min)	0.2
Acquisition End Time (min)	40
Full Scan Range (u)	35–450
Dwell Time (ms)	20
SIM Ions	See Table 3

TRACE GC Ultra	
Oven Method	
Initial Temp (°C)	40
Initial Time (min)	1.0
Rate #1 (°C/min)	5
Initial Temp #2 (°C)	60
Initial Time #2 (min)	1
Rate #2 (°C/min)	3
Initial Temp #3 (°C)	125
Hold Time #3 (min)	1
Rate #3 (°C/min)	10
Final Temp (°C)	238
SSL Method	Splitless
Temperature (°C)	220
Mode	Splitless
Splitless Time	3 min
Carrier Flow (mL/min)	1.2
Gas Saver	On
Vacuum Compensation	On
Transfer Line (°C)	250

Table 2: Instrument method summary for the full scan/SIM analysis of target molecules on the ISQ and TRACE GC Ultra

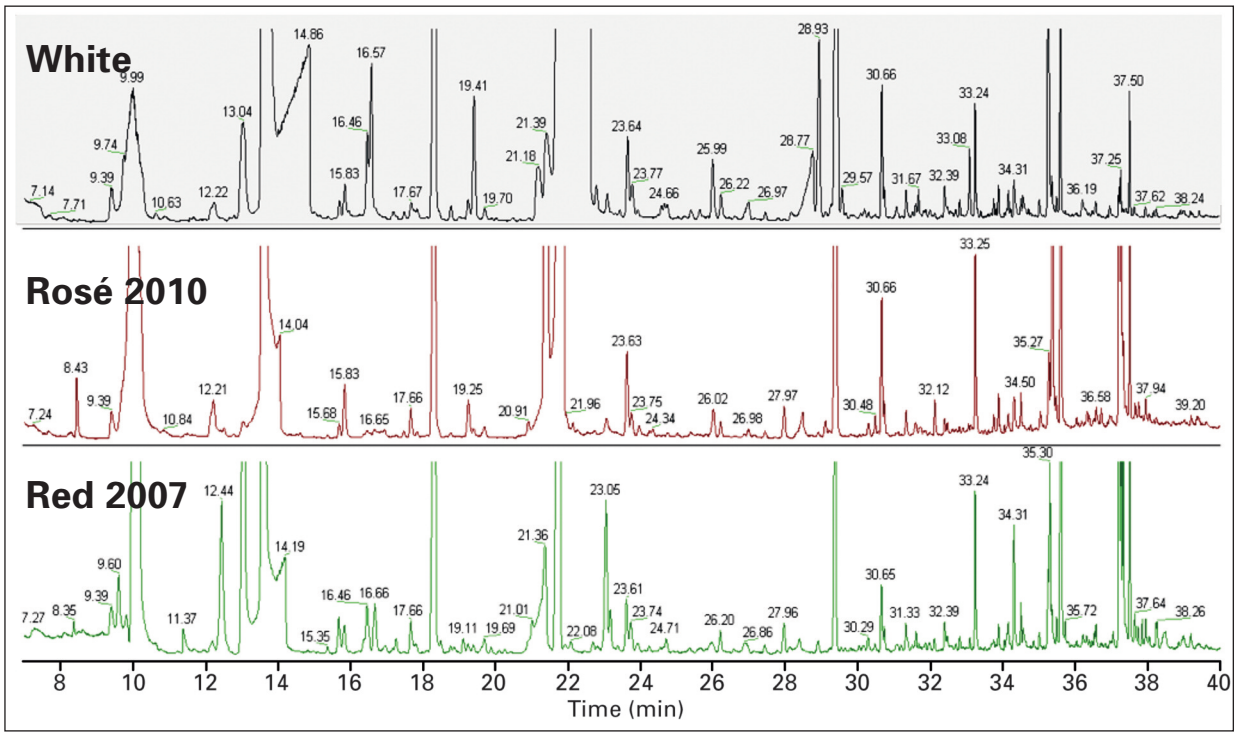

Figure 2: Chromatograms showing full-scan acquisitions for three wine types

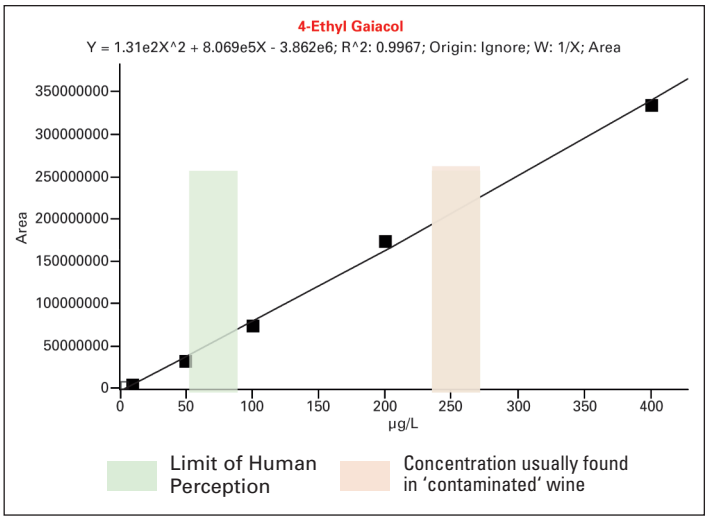

Figure 3: 4-Ethylgaiacol from 50 to 100 µg/L

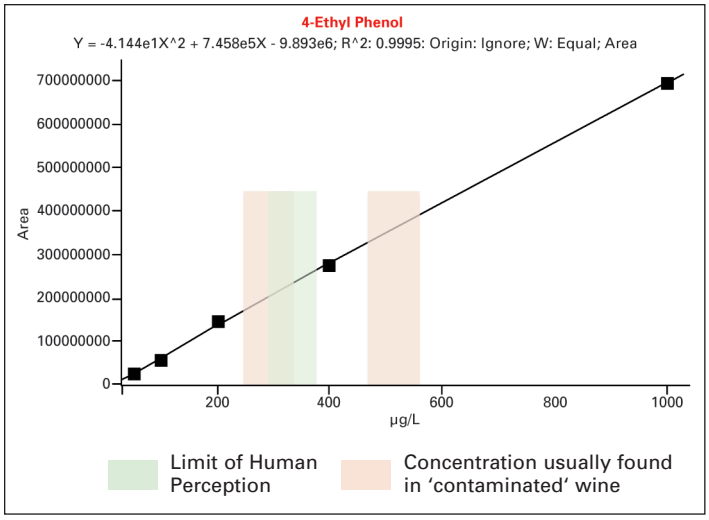

Figure 4: 4-Ethylphenol from 300 to 400 µg/L

Target Molecule	*m/z*
IPMP	124, 137, 152
IBMP	94, 124, 151
4-Ethylphenol	77, 107, 122
4-Ethylgaiacol	122, 137, 153
Trichloroanisole	195, 210, 212
Geosmine	111, 112, 125
Tetrachloroanisole	231, 244, 246
2,4,6-Tribromoanisole	329, 344, 346
Pentachloroanisole	278, 280, 282

Table 3: SIM ions monitored for the target compounds

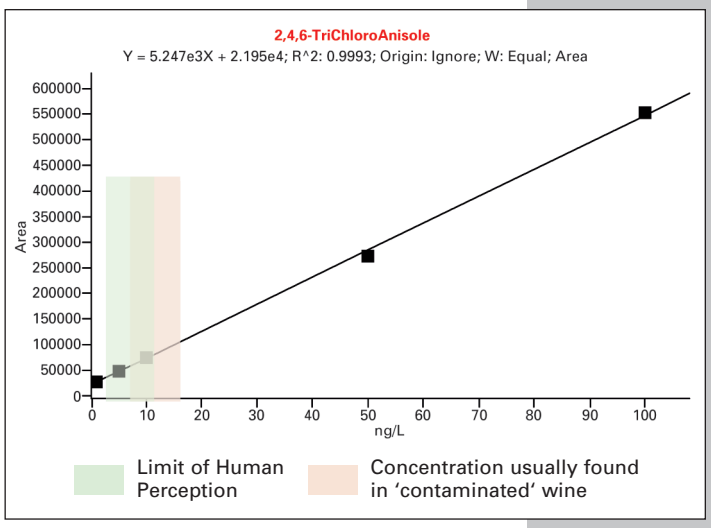

Figure 5: 2,4,6-Trichloroanisole from 2 to 5 ng/L

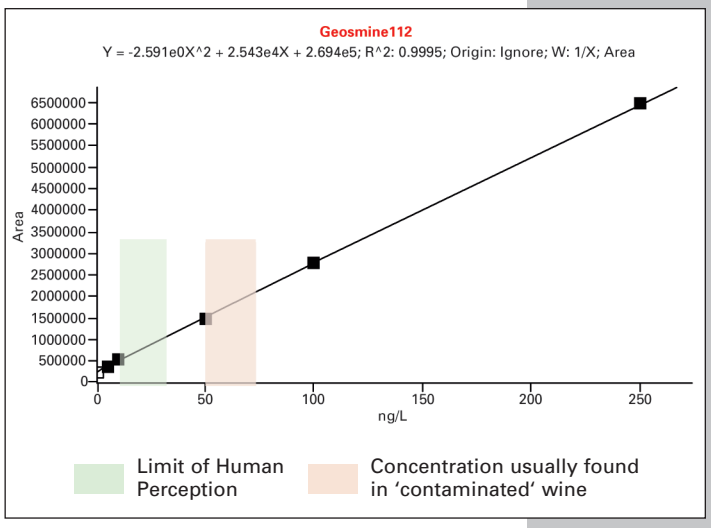

Figure 6: Geosmine from 10 to 50 ng/L

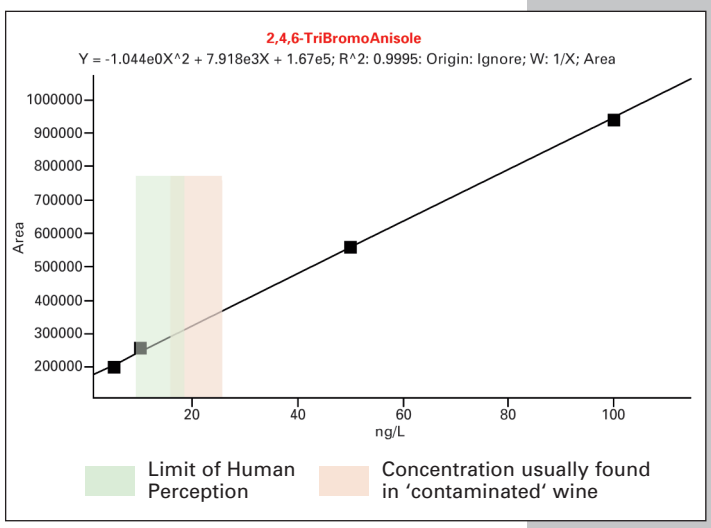

Figure 7: 2,4,6-Tribromoanisole from 10 to 20 ng/L

Conclusion

The ability of the ISQ GC-MS to detect several contaminants in wine at lower concentrations than the limit of human tasters, and its ease of use in combination with a single-step, two-minute sample preparation make it a useful tool for the wine industry. The sequential full-scan/SIM acquisition method for detecting the impurities also does not require extensive training of personnel to provide accurate results. In addition, this general method may be improved or customized to particular wines by incorporating new parameters such as trying other SPME coatings in the extraction phase.

The wine, champagne, and spirit market can be well served by analytical chemistry tools such as GC-MS. There are also other potential uses for this analysis method. For example, wine and other spirit producers risk their recipes being compromised when they outsource their product analysis, and prefer to conduct it on site. In addition, analysis of competitors' products using a GC-MS can help producers quantify what makes one wine superior to another.

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AN52242_E 10/11M