Chemical Analysis of Wine with HS-SPME and GC-TOFMS for Target Screening and Non-Target Characterization and Comparison

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Introduction

Chemical analysis of the aromas associated with wine provides useful information for screening and understanding a product or process. Here, we utilize headspace solid phase micro-extraction (HS-SPME) as a sample preparation method to collect and concentrate volatile analytes in the headspace of a wine sample, followed by gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS). The headspace analytes are concentrated onto the SPME fiber allowing for low level detection. GC then effectively separates analytes within the complex samples for TOFMS detection, which provides identification information through full-mass range library searchable spectra. These techniques offer non-targeted and comprehensive chemical data to describe wine samples that could also be probed for specific targeted compounds.

Food Products and Methods

Commercially available wines sealed with screw caps were used for the wine matrix, so naturally occurring 2,4,6-tricholoranisole (TCA) was not anticipated. For the targeted analysis, TCA (Sigma Aldrich, USA), was spiked into the wine matrix at concentrations ranging from 5 parts-per-trillion (ppt) to 10 parts-per-billion (ppb). For the fresh versus oxidized comparison, a bottle was opened, partially emptied, exposed to air, loosely resealed, and stored at room temperature for roughly two weeks prior to analysis. All samples were prepared for HS-SPME by transferring 10 mL of wine and 3 g of salt into a 20 mL vial sealed with a septum cap. The samples were incubated (5 min) and extracted (30 min) at 65˚C with a 2 cm DVB/CAR/PDMS fiber (Sigma Aldrich). GC conditions are listed in Table 1.

Table 1. Instrument Conditions

Gas Chromatograph	Agilent 7890 with MPS2 Autosampler
Injection	2 min fiber desorption with inlet @ 250°C, splitless
Carrier Gas	He @ 1 ml/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Oven Program	40°C (2 min), ramp 5°C/min to 200°C, ramp 20°C/min to 300°C (1 min)
Transfer Line	260°C
Mass Spectrometer	LECO Pegasus® HT
Ion Source Temp	250°C
Mass Range	33-500 m/z
Acquisition Rate	15 spectra/s

Targeted Screening for TCA

A set of Shiraz wine samples were spiked with 2,4,6-trichloroanisole at ppt to ppb levels to simulate the cork-taint wine fault and to demonstrate the capability of this analytical approach to screen for and quantify this targeted analyte at levels near the sensory threshold.

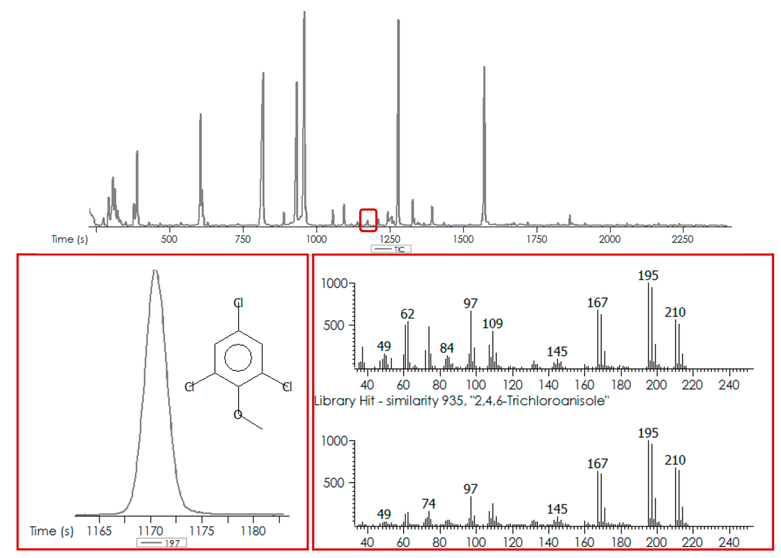


Figure 1. A GC-TOFMS chromatogram for a sample spiked with 1 ppb TCA is shown. Many aroma analytes are observed in the chromatogram including the target (TCA), highlighted in the red box. An XIC (m/z 197) shows the chromatographic peak. The spectral data is also shown with the NIST library match.

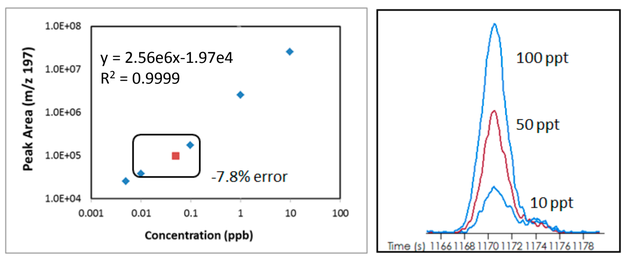


Figure 2. TCA was spiked into the wine matrix at concentrations between 5 ppt and 10 ppb and a calibration was determined with an R^2 value of 0.9999. The calibration equation was applied to a wine sample spiked with 50 ppt TCA that was not included in the calibration. The chromatographic peak profile is shown in red, along with the two bracketing concentrations (10 ppt and 100 ppt) shown in blue. The calculated concentration from the equation agreed with the spiked concentration with an error of less than 8%.

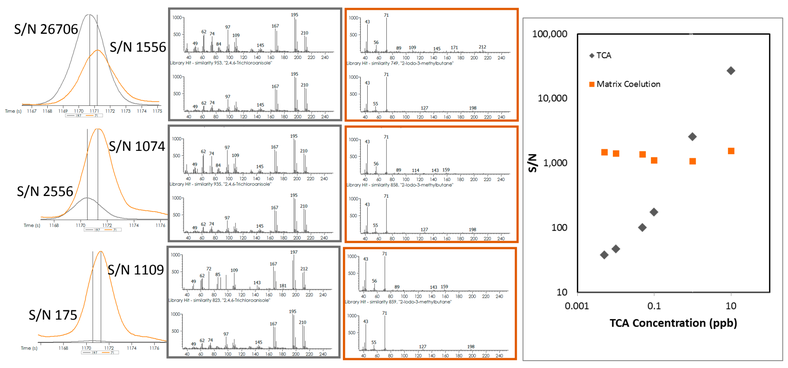
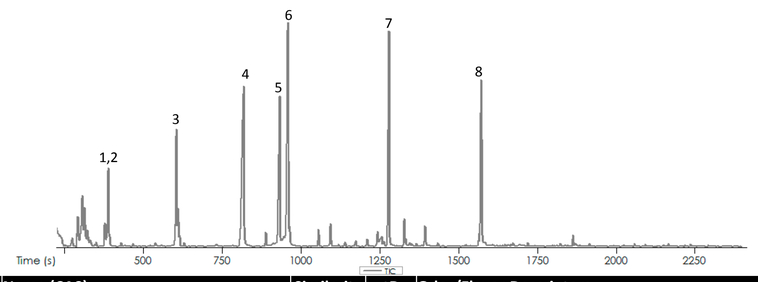


Figure 3. A benefit of this analytical approach is mathematical deconvolution that can handle matrix interferences and coelutions. In this case, the target analyte (TCA), coelutes with an interference from the matrix. ChromaTOF® brand software's automated deconvolution algorithms mathematically separate these coelutions and provide mass spectral and chromatographic peak profile information for both analytes. Deconvolution can accommodate coelutions even when the S/N differs between the analytes, as it does here. The S/N for the matrix analyte is roughly the same in every sample, while the S/N for the target analyte decreases with decreasing concentration, as expected.

Non-targeted Screening

With full m/z range TOFMS acquisition, non-targeted analyses are also possible without additional injections. Utilizing the same data acquired for the targeted screening, general characterization of aroma analytes was performed to gain insight to the overall aroma profile of the wine. Hundreds of analytes were detected and measured in addition to TCA, including a number of volatile and semi-volatile analytes that contribute to the taste and aroma of the wine including esters, carboxylic acids, alcohols, lactones, aromatics (hydrocarbons, phenols, aldehydes, etc.), and various sulfur-containing analytes.



	Name (CAS)	Similarity	tR	Odor/Flavor Descriptors
1	1-Hexanol (111-27-3)	952	383	Fruity, alcoholic, green, apple-skin, sweet, and oily
2	3-Methylbutyl ethanoate (123-92-2)	948	389	Sweet, Fruity, Banana
3	Ethyl hexanoate (123-66-0)	969	605	Sweet, Fruity, pineapple, banana, waxy, green
4	Phenylethyl Alcohol (60-12-8)	953	821	Floral, sweet, rose, bready
5	Ethyl succinate (123-25-1)	950	935	Mild, Fruity, apple
6	Ethyl octanoate (106-32-1)	926	960	Fruity, Wine, waxy, sweet apricot, banana, brandy, pear, pineapple, cognac
7	Ethyl decanoate (110-38-3)	911	1280	Sweet, waxy, fruity, apple grape, oily, brandy
8	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester (74381-40-1)	891	1573	

Figure 4. The most intense peaks in the chromatogram are labeled and identified in the wine sample. The associated odor and flavor descriptions are included for these non-targeted analytes. Hundreds of additional peaks were also found that are likely to contribute to the taste and aroma of the wine.

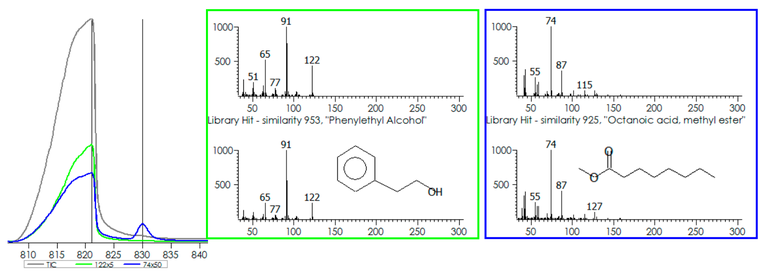


Figure 5. A zoomed view of Peak #4 in Figure 4 is shown here. In the TIC (black trace), only phenylethyl alcohol is apparent, but by viewing other XICs determined from peak finding, additional lower level analytes, like octanoic acid methyl ester, are found (apparent with m/z 74, shown with a 50x zoom).

Non-Targeted Differentiation

Further non-targeted comparisons between fresh and oxidized wine samples were made which determined specific chemical differences.

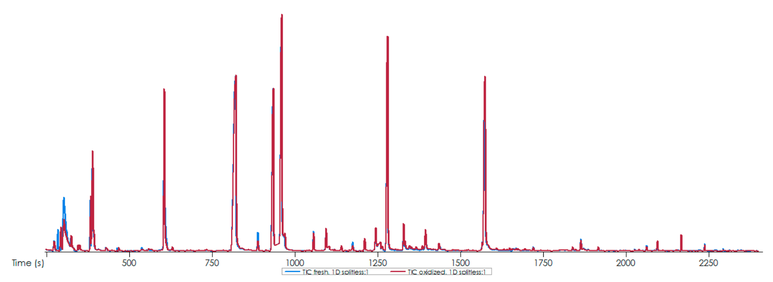


Figure 6. By visual review, the fresh (blue) and oxidized (red) samples appear quite similar because the most intense peaks in each sample are consistent. Differentiating the samples required digging deeper with data processing to uncover analyte peaks that do not stand out in the TIC.

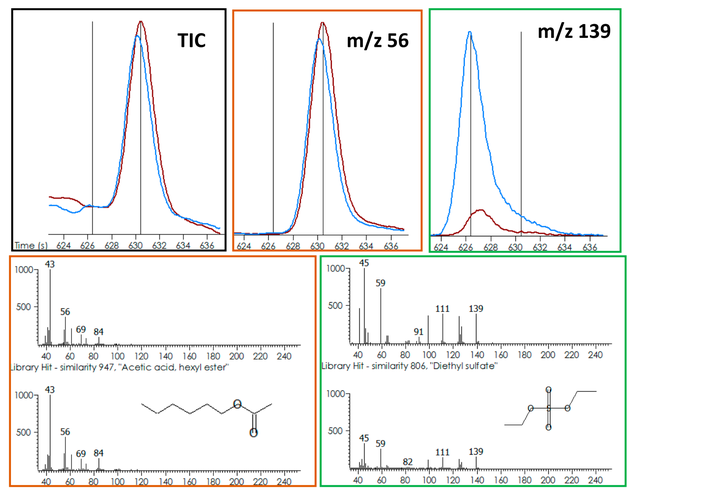


Figure 7. Automated data processing and peak finding facilitate finding these analyte differences. In this example, both low intensity and a coelution obscure the peak that differs between the fresh (blue) and oxidized (red) samples. The peak that is apparent in the TIC, hexyl acetate with fruity odor properties, does not differ between the fresh (blue) and oxidized (red) samples. However, another vertical line peak marker is also visible, and when an m/z specific to that analyte is plotted, a sulfur-containing compound that is present at levels nearly 9-fold higher in the fresh sample relative to the oxidized sample is observed. The differential expression is clear in the XICs and data processing results even though it was hidden in the TIC.

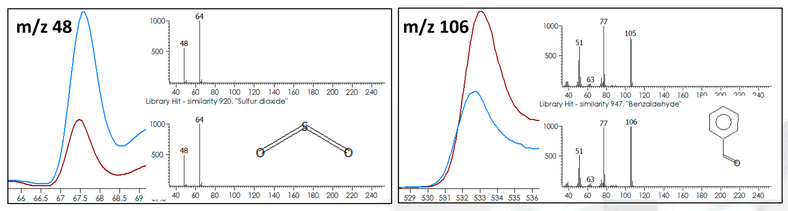


Figure 8. Two more analytes that differ between the samples, apparent in the XICs, are shown here. Sulfur dioxide, which is often added to wine samples as protection against oxidation, was observed nearly 3x higher in the fresh (blue) sample. Benzaldehdye, with a fruity odor, is observed at roughly 2x lower in the fresh sample compared to the oxidized sample (red).

Benefits of Extension to GC×GC

By extending the analytical technique to two-dimensional gas chromatography (GC×GC), additional distinction between the samples could be determined. GC×GC offers increased peak capacity and a lower limit of detection, which led to the detection of more analytes within these complex samples, and the determination of additional chemical differences.

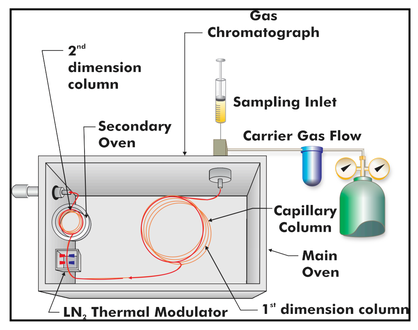


Figure 10. GC×GC couples two columns with complementary stationary phases in series. (Here an Rxi-17Sil MS (0.6 m x 0.25 mm i.d. x 0.25 µm phase coating) was added to the Rxi-5ms). A thermal modulator connects the two columns and effluent from the first is cryogenically focused and injected to the second at set time intervals/modulation periods (2s). The effluent from the second column is introduced to TOFMS which provides full mass range data for identification and quantification.

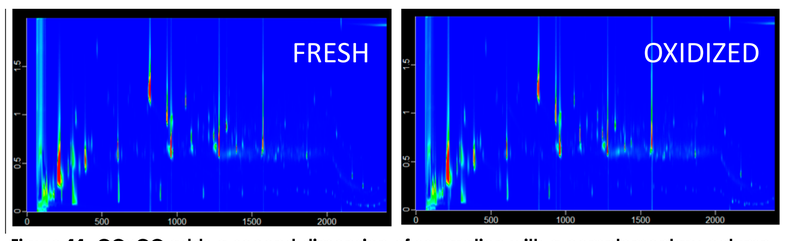


Figure 11. GC×GC adds a second dimension of separation with a complementary column, spreading analytes out into 2D space. Representative chromatograms are shown for each sample.

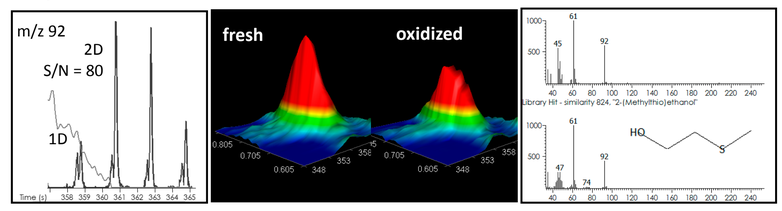
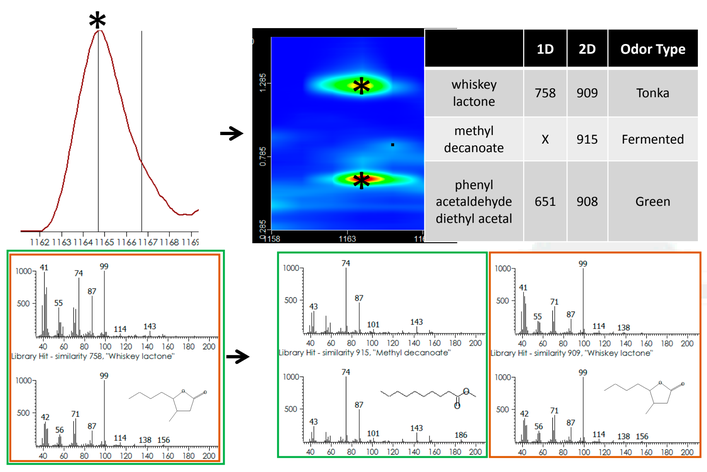


Figure 12. One reason for the increase in number of detected analytes is that GC×GC offers lower limits of detection through thermal focusing at the modulator, just prior to detection. The peak area is maintained, but thermal focusing narrows GC×GC peaks leading to increased peak height and S/N relative to GC. Here, m/z 92 does not show a distinct peak shape in the 1D data, but does in the 2D data. This boost in S/N brought 2-methylthio-ethanol above the S/N threshold in the 2D data. This analyte has a meaty odor and was observed at levels 1.5 higher in the fresh sample.



	1D	2D	Odor Type
whiskey lactone	758	909	Tonka
methyl decanoate	X	915	Fermented
phenyl acetaldehyde diethyl acetal	651	908	Green

Figure 13. Another reason for the increase in number of analytes detected is the improved peak capacity that comes with the complementary second dimension column. Analytes that coelute in the first dimension can sometimes be separated in the second dimension. In some of these cases, deconvolution addressed the coelution, and in other cases the overlap exceeded deconvolution capabilities. The TIC in the 1D data shows only one apparent peak. Deconvolution and the automated data processing determined two analyte peaks were coeluting. In the 2D data, three peaks were chromatographically separated from each other. The spectral information for the first peak marker (indicated with an asterisk) is the combination of the two chromatographically separated peak markers in the GC×GC data, also indicated with asterisks. Improved identifications and information on an additional analyte were achieved with GC×GC.

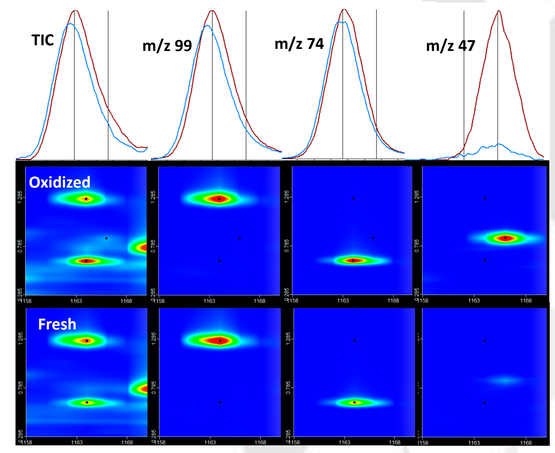


Figure 14. A comparison of the fresh (blue) and oxidized (red) samples in 1D and 2D. The TIC is shown as well as the m/z for the determined analyte (m/z 99 for whiskey lactone, m/z 74 for methyl decanoate, and m/z 47 for phenyl acetaldehyde diethyl acetal). These plots demonstrate both the improved chromatographic separation for whiskey lactone and diethyl acetal, and the differential expression (if present) for the analytes.

Conclusions

This study demonstrates the ability to detect a target analyte (2,4,6-tricholoranisole), at parts-per-trillion levels within a wine matrix. TCA is attributed to the cork-taint wine fault, and these detected concentrations are comparable to typical sensory thresholds. Calibration data were demonstrated and applied to a known spiked sample with good accuracy, even in the presence of a matrix coelution. The same data were also reviewed in a non-targeted way to generally characterize a sample and to differentiate and distinguish related samples. A fresh and oxidized wine sample were analyzed and appeared quite similar based on the TIC view of the samples. Peak finding and deconvolution uncovered specific analytes that differed between the samples that were not apparent in the TIC. The further addition of GC×GC uncovered specific analytes that differed between fresh and oxidized that were either below the S/N threshold or confounded by coelutions in the 1D data.

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