Differentiation of Fresh and Oxidized Wine Samples with HS-SPME, GC-TOFMS, and GC×GC-TOFMS

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Key Words: HS-SPME, GC-TOFMS, GC×GC-TOFMS, Pegasus HT, Pegasus 4D, Deconvolution, Wine Aroma

1. Introduction

Chemical analysis of the aromas associated with wine provides useful information for understanding a product or process. Proper storage and the impact of the introduction of oxygen to a wine sample are of interest and are explored further here. Two bottles of wine, one stored properly and one that was intentionally oxidized from improper storage, were compared with headspace solid-phase micro-extraction (HS-SPME) as a sample preparation method to collect and concentrate volatile analytes from the headspace of wine samples. Chemical analysis with gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) and two-dimensional GC-TOFMS (GC×GC-TOFMS) were then performed. Both analytical techniques offer non-targeted and comprehensive chemical data for the samples that help you see what you are missing and characterize the samples. The extension to GC×GC with the second complementary separation dimension provides additional distinction between the samples due to the increased peak capacity and lower limit of detection. These benefits provided the ability to detect more analytes within these complex samples and uncover additional chemical differences between the storage conditions.

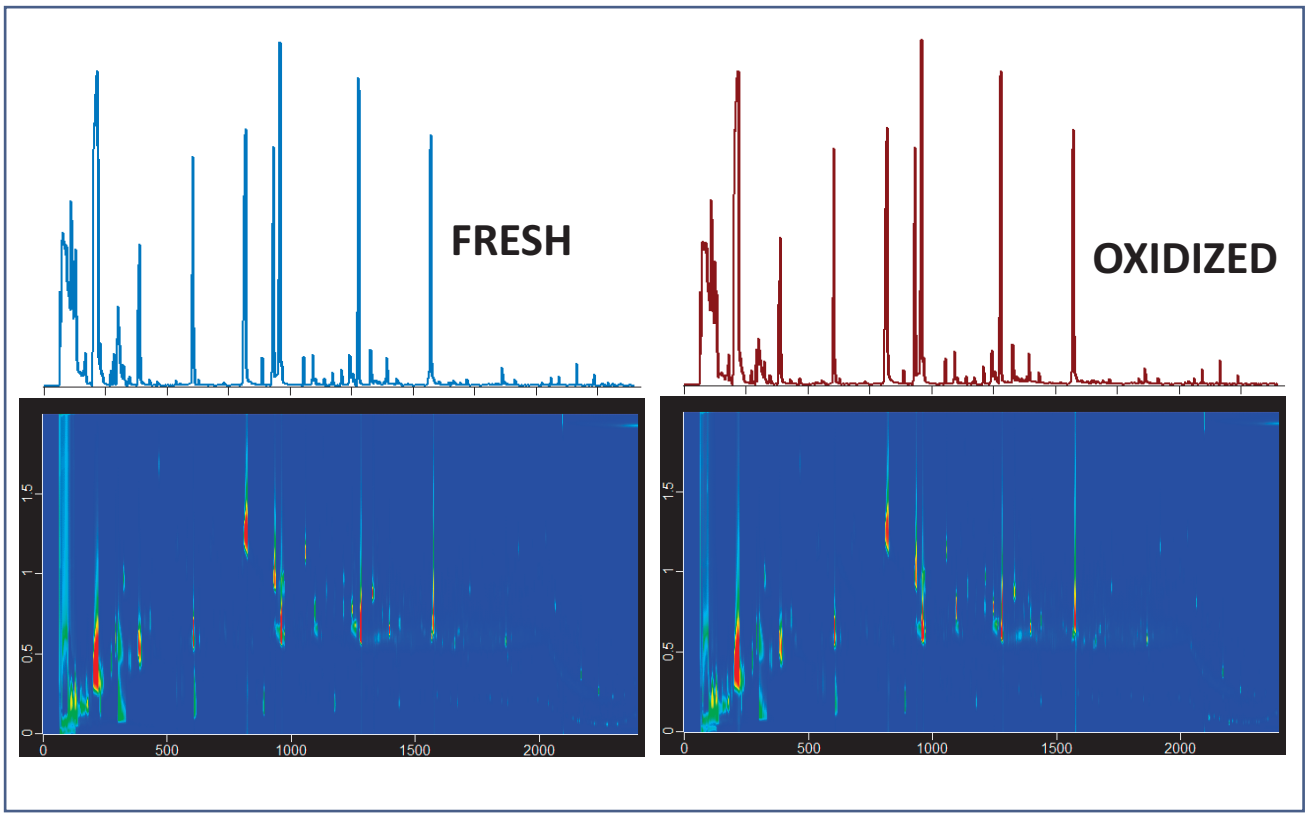

Figure 1. GC-TOFMS (top) and GC×GC-TOFMS (bottom) chromatograms for a fresh and oxidized wine sample are shown above. Both analytical approaches provide good characterization of the samples, but differentiation is difficult by visual review alone. The most intense peaks in each sample appear quite similar.

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2. Experimental

Two bottles of the same commercially-available wine were acquired. One bottle was opened, partially emptied and exposed to air, then loosely resealed and stored at room temperature for roughly two weeks prior to analysis. The second bottle was opened and analyzed fresh the day of analysis. All samples were prepared for HS-SPME by transferring 10 mL of wine and 3 g of salt into a 20 mL vial and sealing with a septum cap. The samples were incubated (5 min) and extracted (30 min) at 65°C. Extraction was performed with a 2 cm DVB/CAR/PDMS fiber (Sigma Aldrich) which was then exposed in the GC inlet for analysis with conditions listed in Tables 1 and 2.

Table 1. GC-TOFMS (Pegasus® HT) 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	2 min at 40°C, ramped 5°C/min to 200°C, ramped 20°C/min to 300°C held 1 min
Transfer Line	260°C
Mass Spectrometer	**LECO *Pegasus* HT**
Ion Source Temperature	250°C
Mass Range	33-500 m/z
Acquisition Rate	15 spectra/s

Table 2. GC×GC-TOFMS (*Pegasus* 4D) 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 One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Column Two	Rxi-17 Sil MS, 0.6 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Oven Program	2 min at 40°C, ramped 5°C/min to 200°C, ramped 20°C/min to 300°C held 1 min Secondary oven maintained +10°C relative to primary oven
Modulation	2 s with temperature maintained +15°C relative to 2nd oven
Transfer Line	260°C
Mass Spectrometer	**LECO *Pegasus* HT**
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	200 spectra/s

3. Results and Discussion

The fresh and oxidized samples were each analyzed by GC and GC×GC for general characterization through chemical analysis. Chromatograms for each sample and with each analytical technology are shown in Figure 1. Both approaches provided information on 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. By visual review, the fresh and oxidized samples appear quite similar because the most intense peaks in each sample are consistent. Differentiating the samples requires digging deeper with ChromaTOF® brand software's data processing tools to uncover analyte peaks that do not stand out in the TIC. In some cases, the peak intensity is low and XICs are needed to view the peak, and in other cases analyte coelutions obscure the peak that differs and mathematical deconvolution is needed.

Figure 2 shows an example where both low intensity and a coelution obscure the peak that differs between the fresh and oxidized 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. This sample distinguishing analyte needed data processing and deconvolution to be observed. Two other examples of analytes that were determined through peak finding that differ between the samples are shown in Figure 3.

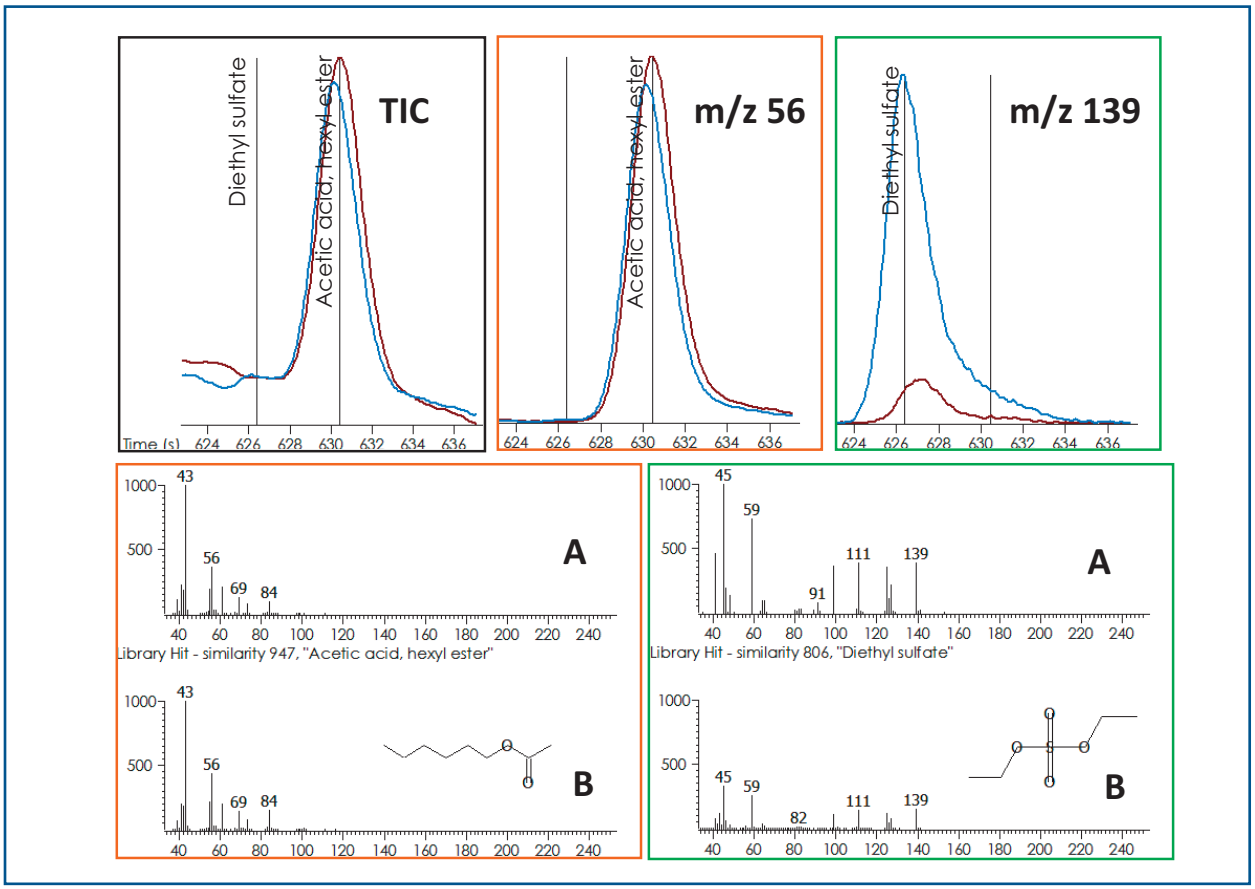


Figure 2. The fresh (blue) and oxidized (red) samples do not appear different in the TIC chromatograms that are overlaid here. Two peaks were determined with automated data processing and deconvolution, and when XICs for each are plotted, the unique chromatographic peak shapes are apparent. Hexyl acetate (m/z 56, fruity odor type) is present at the same levels in each sample, while diethyl sulfate (m/z 139) is present at nearly 9x higher in the fresh sample. The differential expression is clear in the XICs even though it was hidden in the TIC. The Peak True (deconvoluted) mass spectra (A) for these analytes are shown along with their NIST library match (B).

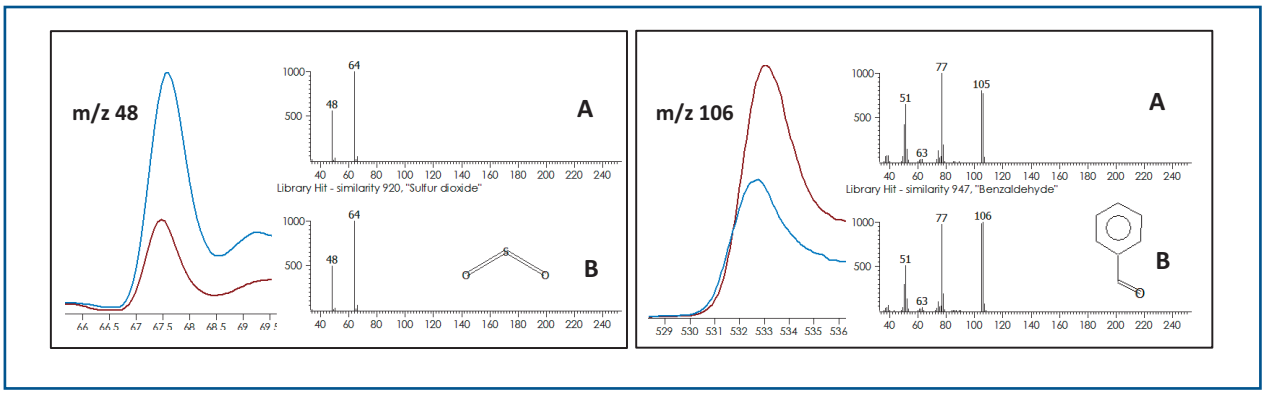


Figure 3. Two other analytes that differ between the samples are shown here. Sulfur dioxide, which is often added to wine samples as protection against oxidation, was observed nearly 3x higher in the fresh sample. Benzaldehdye with a fruity odor is observed at roughly 2x lower in the fresh sample compared to the oxidized sample. Automated data processing and peak finding facilitate finding these analyte differences and provided deconvoluted spectra (A) that were compared to NIST libraries (B) for identification.

The analytical capabilities can be extended by adding a second dimension of separation with GC×GC. This approach brings additional benefits that usually result in a higher number of detected peaks. One reason for the increase with GC×GC is that there is an enhanced S/N gained through thermal focusing at the modulator. Effluent is thermally trapped and focused for injection to the second column which sharpens analyte peaks just prior to detection increasing the S/N. An example is shown in Figure 4. This sulfur-containing analyte was detected and differentially expressed in the 2D data, but not detected in the 1D data.

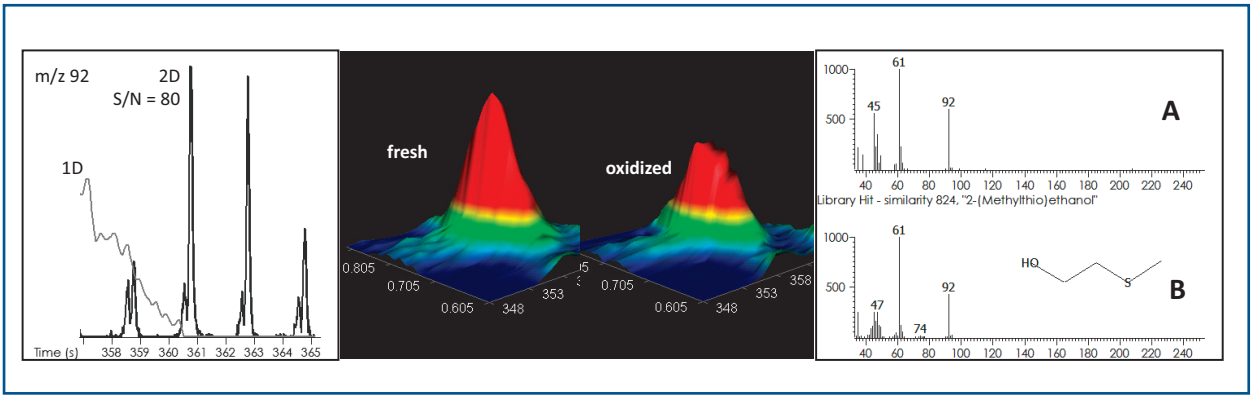
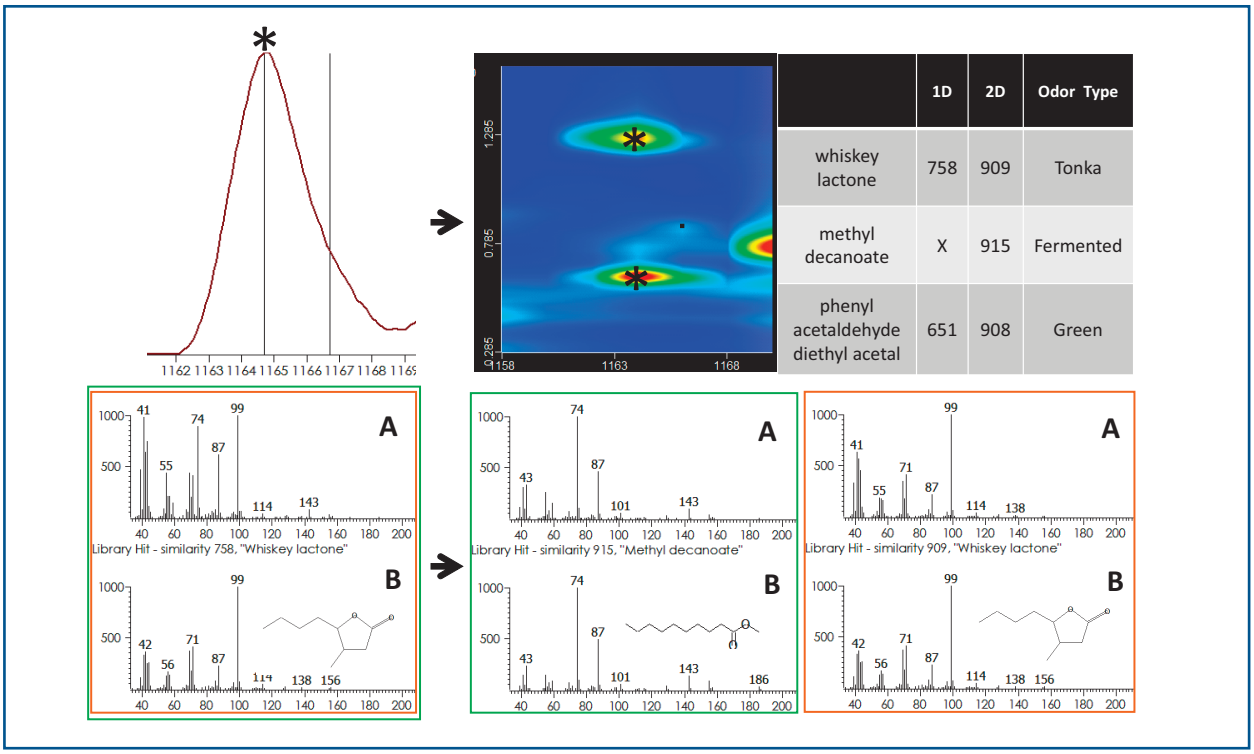


Figure 4. Thermal focusing enhances the S/N for GC×GC data. m/z 92 does not show a distinct peak shape in the 1D data, but does in the 2D data. This boost in S/N brought 2-methylthio-ethanol above the S/N threshold in the 2D data. This analyte has a meaty odor and was observed at levels 1.5 higher in the fresh sample. The Peak True spectrum (A) is shown along with the NIST library match (B).

Another reason for the increase in 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. An example is demonstrated in Figures 5 and 6.



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

Figure 5. 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. The Peak True (deconvoluted) mass spectra (A) for these analytes are shown along with their NIST library match (B).

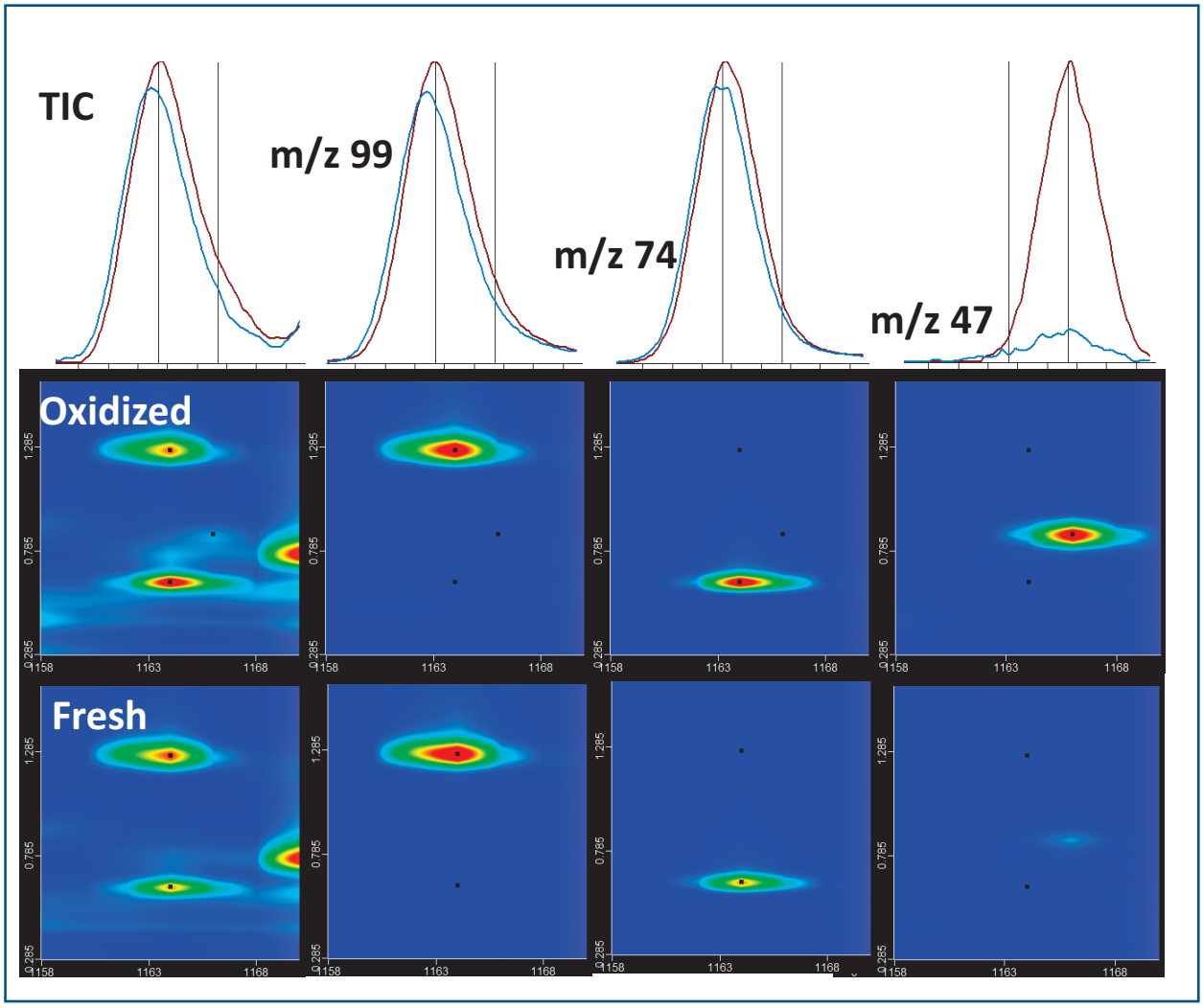


Figure 6. A comparison of the fresh (blue) and oxidized (red) samples in 1D and 2D. The TIC is shown as well as the m/z for the determined analyte (m/z 99 for whiskey lactone, m/z 74 for methyl decanoate, and m/z 47 for phenyl acetaldehyde diethyl acetal). These plots demonstrate both the improved chromatographic separation for whiskey lactone and diethyl acetal, and the differential expression (if present) for the analytes.

4. Conclusion

This study demonstrates LECO's analytical platform and data analysis tools to differentiate and distinguish related samples. A fresh and oxidized wine sample was 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. Pegasus HT full mass range sensitivity and speed with unparalleled deconvolution capabilities allows you to see more in a standard analysis. The addition of GC×GC (Pegasus 4D) allows you to confidently discover even more analytes to see what you've been missing.

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