Analysis of Trichloroanisole in Wine and Cork using a Purge and Trap Multimatrix Autosampler

Application Note

Abstract

Due to its low odor threshold, 2,4,6-trichloroanisole (TCA), the compound responsible for “cork taint”, requires extremely sensitive analysis. TCA is a fungal metabolite of 2,4,6-trichlorophenol (TCP). Even minute contamination can cause issues with regards to taste. This study will demonstrate a purge and trap method for analyzing wine and cork samples at the part-per-trillion (ppt) level utilizing an in-vial purge. A Teledyne Tekmar Atomx Automated VOC Sample Prep System will be used, employing a soil method to prevent matrix issues associated with analyzing the difficult matrices of wine and cork.

Introduction

Wine that is “tainted” by 2,4,6-trichloroanisole is characterized by its musty or moldy odor. TCA is derived from 2,4,6-trichlorophenol (TCP) after undergoing ortho-methylation[1]. TCP has various uses as a herbicide and pesticide, as well as a wood preservative. The USEPA has classified TCP as a Group B2 substance, a possible human carcinogen. This compound can also form during the chlorination process used to clean industrial wastewater and drinking water[2]. TCA, which is nontoxic, is formed through *ortho*-methylation of these TCP precursors as a natural detoxification reaction caused by different microbes[1]. Although nonhazardous, TCA, as well as other odor compounds, have an impact on the wine industry due to their effect on taste and perception of quality.

This study utilizes an Atomx multimatrix autosampler integrated with a purge and trap concentrator. This set-up allows for complete automation of sample preparation for the analysis of liquid, soil and methanol extracted samples for purge and trap. For the analysis, samples were loaded into the 80-position tray and prepared for extraction. Using an in-vial purge, analytes are purged from of the sample with helium and onto a sorbent trap. The trap is then heated and analytes are desorbed to the GC/MS for analysis. Utilizing an Agilent 7890A/5975 GC/MS in Selective Ion Monitoring (SIM) mode, a linear calibration was performed and percent relative standard deviation (%RSD), method detection limits (MDLs), and percent carryover were determined for TCA. An example of a SIM scan for a 10ppt TCA standard can be found in Figure 1.

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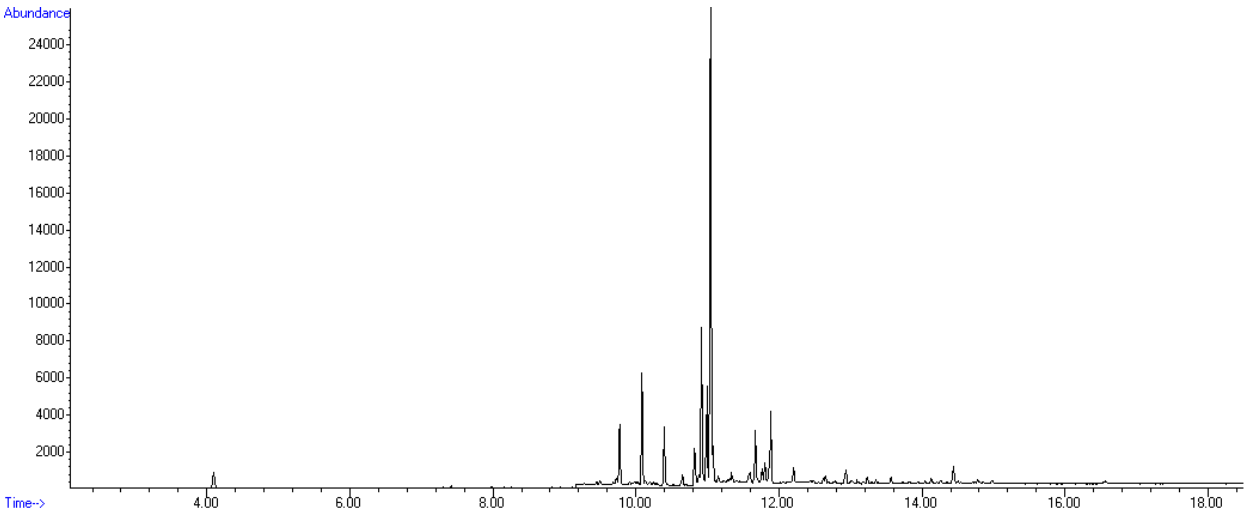

Figure 1: SIM Scan of 10ppt 2,4,6-Trichloroanisole Standard

Experimental-Instrument Conditions

The Atomx Automated VOC Sample Prep System was coupled to an Agilent 7890/5975 GC/MS for this analysis. A Tenax (#1) trap was the analytical trap of choice. The GC was configured with a J&W DB-624 20m x 0.18mm x 1.0µm column. Illustrations 1 and 2 demonstrate the Purge and Desorb flow paths of the Atomx. The GC/MS parameters are outlined in Tables 1 and 2. Table 3 outlines the Atomx conditions.

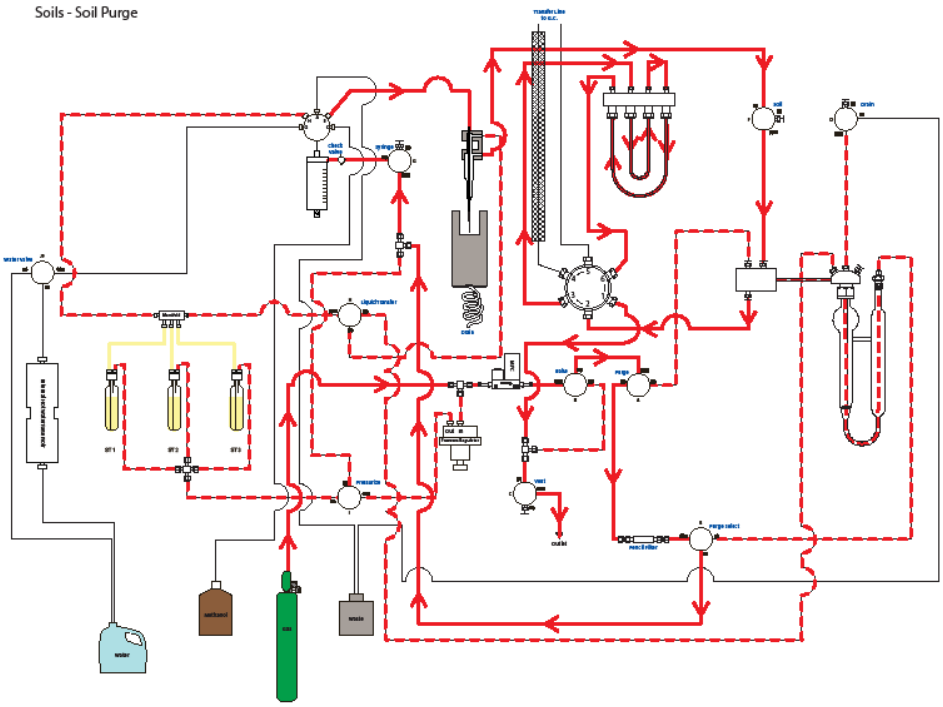

Illustration 1: Atomx Soil Purge Flow Path

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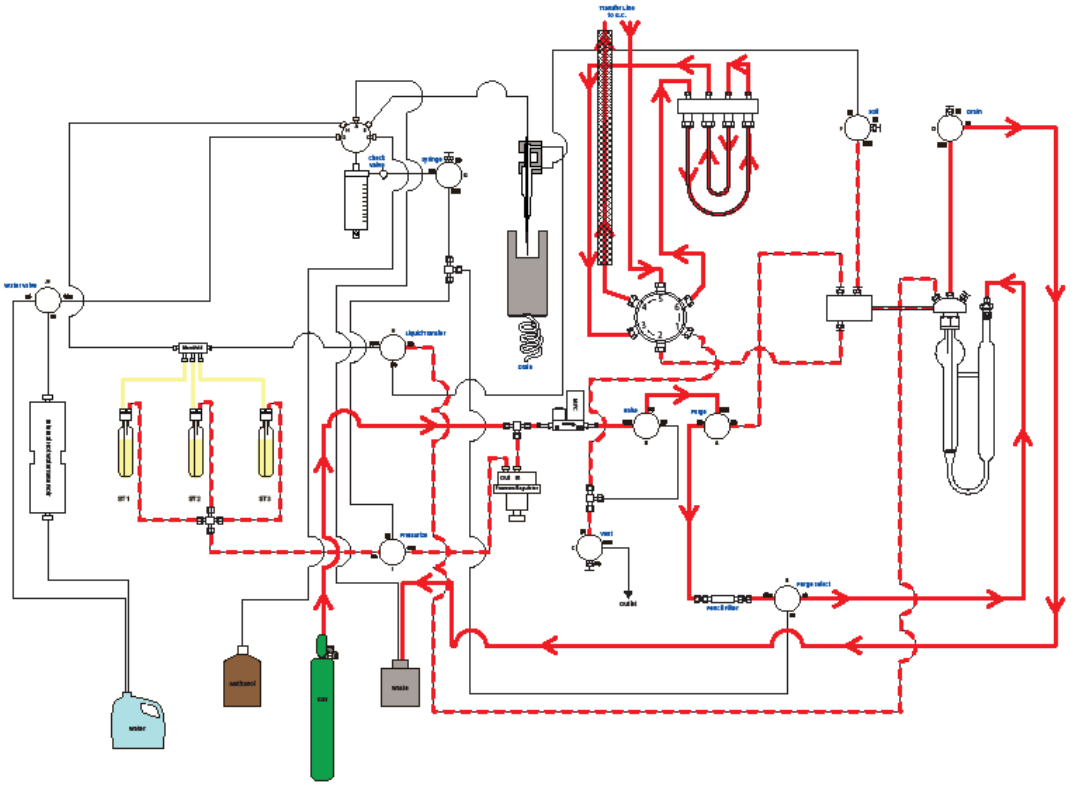

Illustration 2: Atomx Desorb Flow Path

GC Parameters	
GC:	Agilent 7890A
Column:	J&W DB-624 20m x 0.18mm x 1.0µm
Oven Program:	40° C for 2 min, to 160° C at 16° C/min, for 0 min, to 240° C at 20° C/min
Inlet:	220° C
Column Flow:	0.9mL/min
Gas:	Helium
Pressure:	21.542 psi
Split Ratio:	10:1

MS Parameters	
MSD:	Agilent 5975C
Source:	230° C
Quad:	150°C
Solvent Delay:	2.0 min
SIM Ions:	95, 107, 112, 124, 125, 137, 151, 152, 195, 197, 212
Dwell Time:	100msec per ion
MS Transfer Line Temp:	230°

Tables 1 & 2: GC and MSD Parameters

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Atomx Soil Parameters			
Variable	**Value**	**Variable**	**Value**
Valve Oven Temp	175°C	Purge Time	10.00 min
Transfer Line Temp	175°C	Purge Flow	100mL/min
Sample Mount Temp	60°C	Purge Temp	20°C
Water Heater Temp	90°C	Condensate Purge Temp	20°C
Sample Vial Temp	40°C	Dry Purge Time	5.00 min
Prepurge Time	0.00 min	Dry Purge Flow	45mL/min
Prepurge Flow	0mL/min	Dry Purge Temp	20°C
Preheat Mix Speed	Slow	Methanol Needle Rinse	Off
Sample Preheat Time	0.00 min	Methanol Needle Rinse Volume	3.0mL
Soil Valve Temp	100°C	Water Needle Rinse Volume	7.0mL
Standby Flow	45mL/min	Sweep Needle Time	0.25min
Purge Ready Temp	40°C	Desorb Preheat Time	220°C
Condensate Ready Temp	45°C	GC Start Signal	Start of Desorb
Presweep Time	0.25 min	Desorb Time	2.00 min
Water Volume	10.0mL	Drain Flow	300mL/min
Sweep Water Time	0.25 min	Desorb Temp	225°C
Sweep Water Flow	100mL/min	Bake Time	5.00 min
Sparge Vessel Heater	Off	Bake Flow	250mL/min
Sparge Vessel Temp	20°C	Bake Temp	230°C
Purge Mix Speed	Medium	Condensate Bake Temp	200°C

Table 3: Atomx Parameters (items in yellow were not used)

Calibration and Results

A 50ppb stock standard of TCA was prepared in methanol. Calibration standards were prepared in volumetric flasks filled with 10% (w/v) sodium chloride in de-ionized water over a range of 1ppt to 100ppt. Samples were transferred to headspace free 40mL VOA vials for analysis. The Internal Standard (IS), isopropylmethoxypyrazine (IPMP), was prepared in methanol at a 50ppb concentration. After transferring to the standard vessel on the Atomx, the IS was added in 5µL aliquots to each sample, bringing the final concentration of 50ppt, factoring in the 5mL sample volume. Agilent Chemstation software was used to process the calibration data. Relative response factors were evaluated for %RSD and coefficient of determination (r^2) with results for all compounds listed in Table 4. Calibration curves can be found in Figure 2. Method detection limits (MDL) were also established for all by analyzing seven replicates at a concentration of 2ppt. MDL results were below 1ppt. Percent carryover compound was determined by running blank samples after a 1ppb standard.

Compound Name	Average RRF	%RSD	r^2	Minimum Detection Limit	% Carryover
Isopropylmethoxypyrazine (IPMP) (IS)	1.000	N/A	N/A	N/A	N/A
2,4,6-Trichloroanisole	0.644	8.42	0.9996	0.13	0.53

Table 4: Calibration Data for 2,4,6-Trichloroanisole

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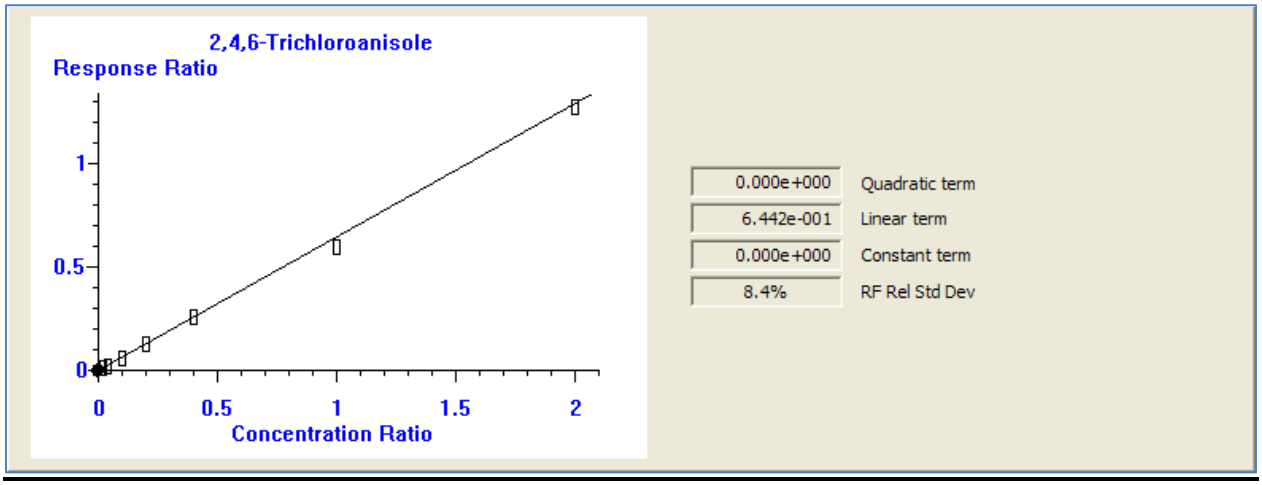

Figure 2: Calibration Curve Data for 2,4,6-Trichloroanisole

This method was also evaluated by examining wine and cork samples to gauge TCA concentrations in real world samples. While the wine samples did not contain any TCA, all cork samples examined in this study had concentrations in the ppt range. Examples of TIC and SIM chromatograms can be found in Figures 3 and 4 for wine and Figures 5 and 6 for cork. Figure 7 shows a cork sample with a concentration of 36.27ppt of TCA in 1g of cork (181.35ppt when factoring dilution). Matrix spikes were also performed by adding 50ppt of TCA to wine samples. Spike recoveries fell between 90 and 110% for three replicates. An example of a matrix spike can be found in Figure 8.

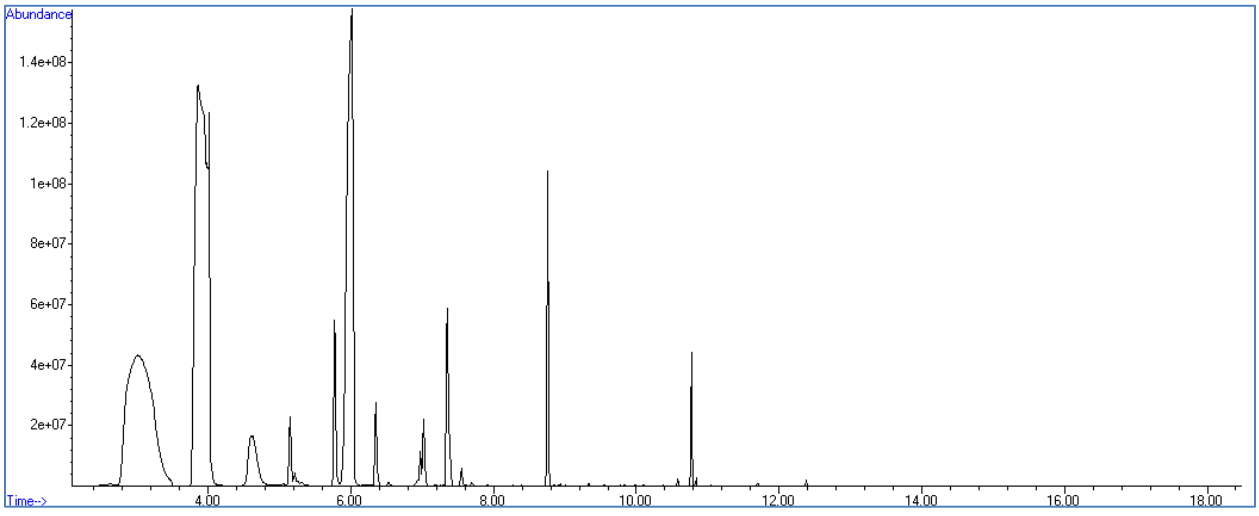

Figure 3: TIC of Wine Sample

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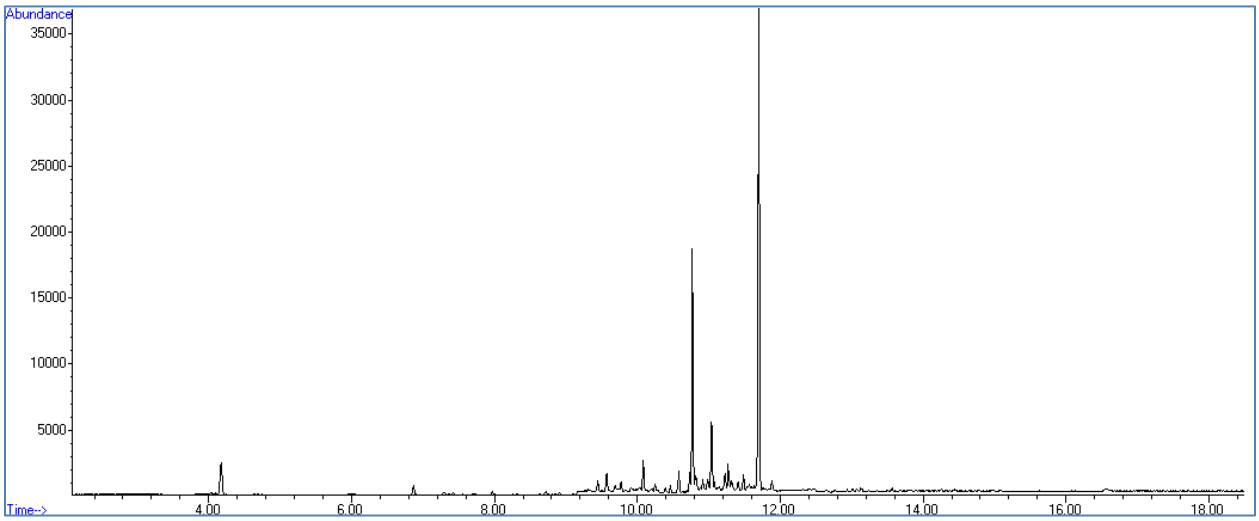

Figure 4: SIM Scan of Wine Sample

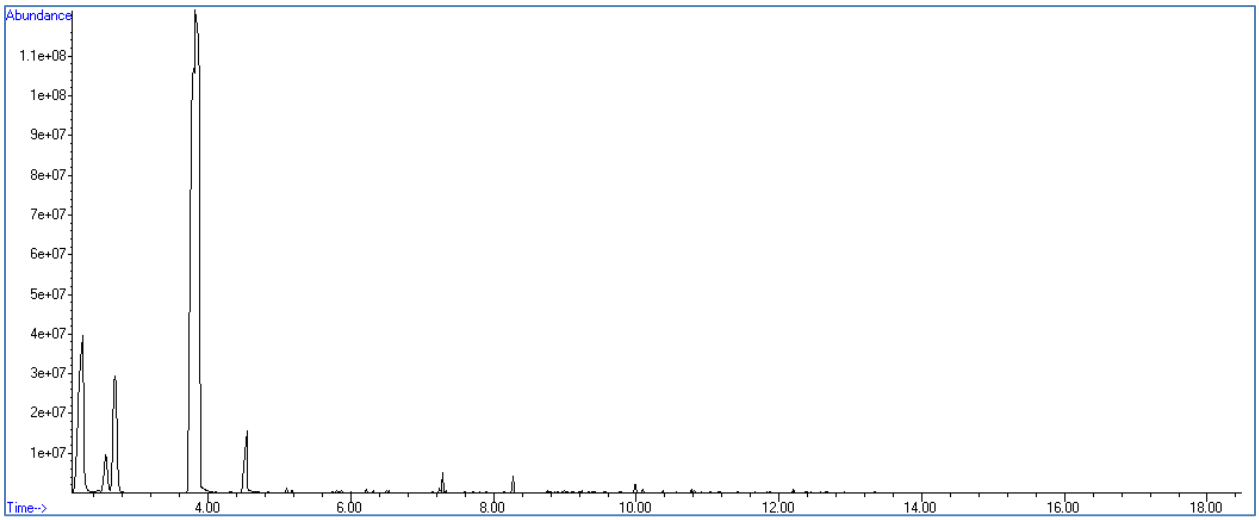

Figure 5: TIC of Cork Sample

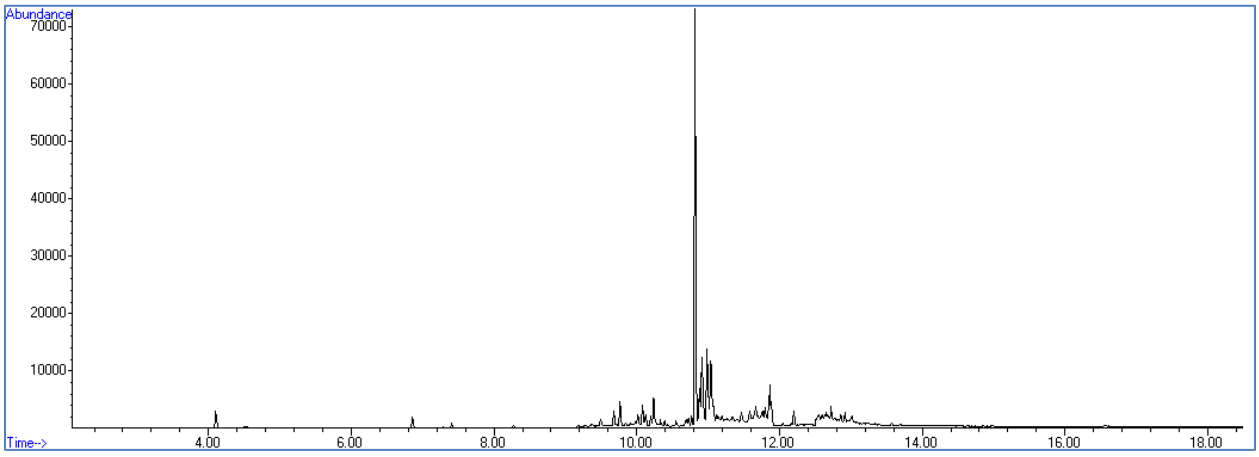

Figure 6: SIM Scan of Cork Sample

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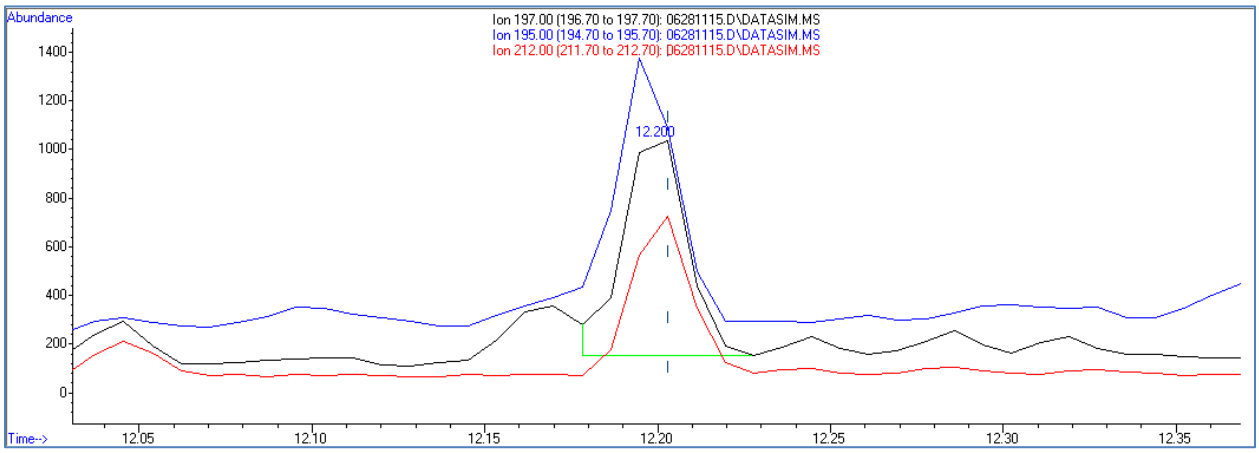

Figure 7: 2,4,6-Trichloroanisole in a Cork Sample (1 gram)

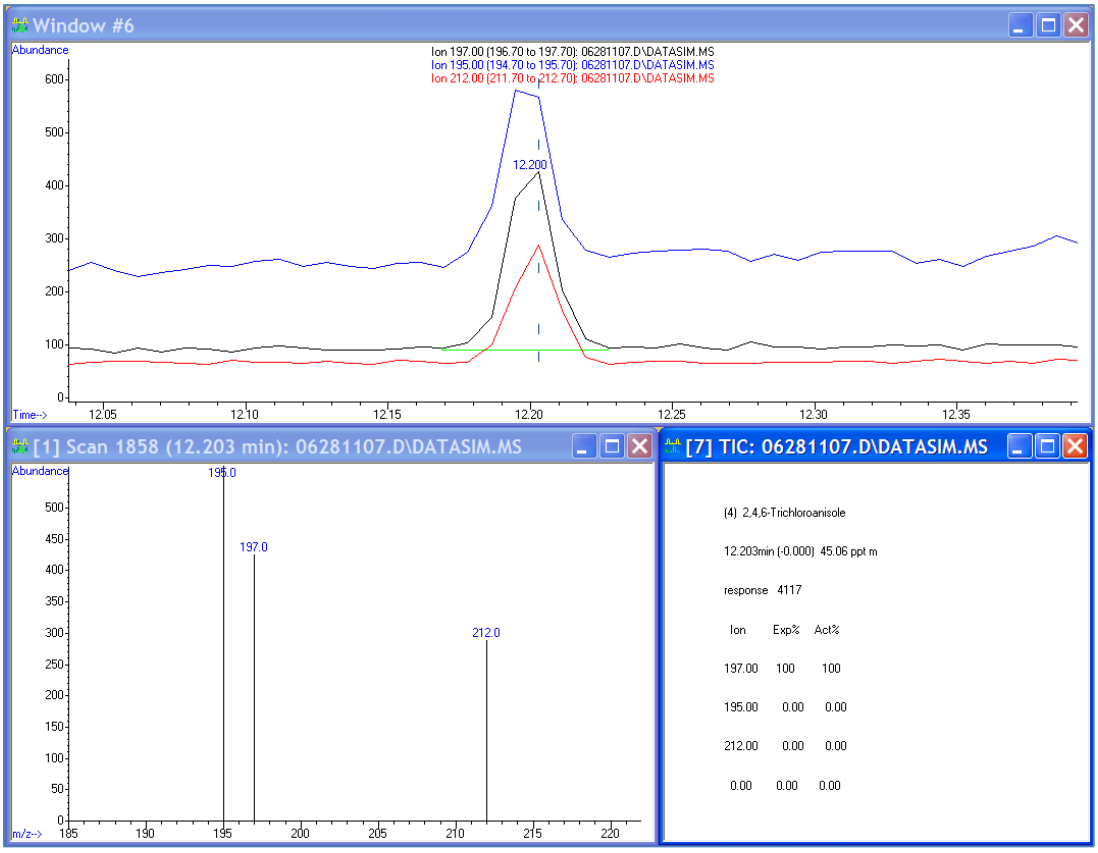

Figure 8: Spike Recovery (50ppt) in Wine Sample

Conclusions

Through the method developed for this analysis using the Atomx, detection limits were established well below human sensory thresholds. Even with complete automation, the precision and accuracy required to detect 2,4,6-trichloroanisole (TCA) at the part-per-trillion level was not sacrificed. Using the in-vial purge employed during a soil method, any issues associated with the difficult matrices of wine and cork are avoided. By completely automating the sample preparation, without compromising sensitivity, efficiency and throughput can be greatly increased while saving time and money.

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References

1. “Cork Taint of Wines: Role of the Filamentous Fungi Isolated from Cork in the Formation of 2,4,6-Trichloroanisole by O Methylation of 2,4,6-Trichlorophenol,” Alvarez-Rodriguez, Lopez-Ocana, Lopez-Coronado, Rodriguez, Martinez, Larriba, and Coque, *Applied and Environmental Microbiology*, Dec. 2002.
2. "2,4,6 Trichlorophenol". *United States Environmental Protection Agency*. Jan 2000. http://www.epa.gov/ttn/atw/hlthef/tri-phen.html

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