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Ultra-trace Analysis of 2,4,6-Trichloroanisole in White Wine Using Automated Solid Phase Microextraction (SPME) and the Varian 240-MS Ion Trap Mass Spectrometer and V:Results™ GC/MS software.

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Introduction

Cork taint in wine is primarily due to the presence of 2, 4, 6-trichloroanisole (TCA). Cork taint is a broad term referring to a set of undesirable smells or tastes found in a bottle of wine, which can significantly affect the wine quality. The formation of TCA in wine is complex, but the most common route is through the conversion of chlorophenols to chloroanisoles (Figure 1) by common microscopic fungi, such as *Aspergillus* sp. and *Penicillium* sp., that are present in the winery environment[1].

2,4,6-Trichlorophenol has been used in various pesticide formulations and also as a wood preservative. Most uses of trichlorophenol have been limited recently in the United States due to health concerns[2]. However, trichlorophenol can form when industrial wastewater containing phenol is treated with hypochlorite, or during the chlorination of drinking water sources. The uptake of a minute amount of chlorophenol by cork tree bark during any stage of its growth, or subsequent uptake in the manufacture of the cork or other stages of winemaking can cause undesirable cork taint.

The human olfactory threshold for detecting TCA is extremely low, near the one part per trillion (ppt or ng/L) level. The quantitative level of TCA detectable using typical single quadrupole instruments was reported to be around 1 ng/L or higher in Selected Ion Monitoring (SIM) mode[3], [4]. In this study, an EI MS/MS method was developed using Solid Phase Microextraction (SPME) and a Varian 240-MS Ion Trap mass spectrometer to measure 2,4,6-trichloroanisole quantitatively in white wine, at levels nearly ten times lower than the human olfactory threshold. This improved limit of detection (LOD) gives winemakers more confidence in their data by avoiding potential false positive or negative results due to matrix interference.

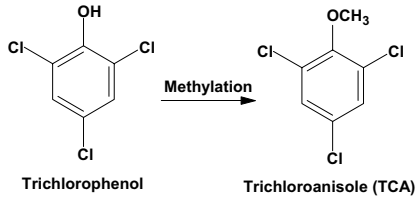

Figure 1. Formation of 2, 4, 6-trichloroanisole.

Instrumentation

- Varian 240-MS Ion Trap Mass Spectrometer
- Varian 450-GC Gas Chromatograph
- Combi PAL AutoSamper with fiber module
- Varian 1177 S/SL (Split/Splitless) injector with a Merlin Microseal™
- V:Results GC/MS software

Materials and Methods

Stock standard solutions and calibration

A 20 µg/L stock solution of 2, 4, 6-trichloroanisole and 2, 4, 6-trichloroanisole-d5 were purchased from Alltech (Nicholasville, KY). White wine free of 2, 4, 6-trichloroanisole was used to prepare calibration standards. The concentrations of the standards ranged from 0.1 to 20 ng/L (0.1, 0.5, 1, 5, and 20 ng/L) with 20 ng/L of 2, 4, 6-trichloroanisole-d5 added as an internal standard.

Sample preparation and extraction

The white wine (10 mL) spiked with various concentrations of TCA and TCA-d5 were transferred to 20 mL headspace vials with 1 g of sodium sulfate. A 100 µm polydimethylsioxane (PDMS) SPME fiber was exposed to the headspace of the sample at 50 °C for 30 min with agitation speed of 500 rpm. Volatile compounds absorbed on the SPME fiber were thermally desorbed at 250 °C for 3 min into an injection port. The fiber was baked out in a bake station at 260 °C for 5 min after each injection.

GC/MS

A Varian 450-GC equipped with a 240-MS Ion Trap mass spectrometer and Combi PAL AutoSamper with fiber module were used (Figure 2). TCA and TCA-d5 were detected and quantified by mass spectrometry in the EI/MS/MS operation mode through internal ionization.

GC Conditions

Column:	FactorFour™ VF-5ms, 30 m x 0.25 mm x 0.25 µm (Part No. CP8944)
Program:	60 °C, programmed to 320 °C at 20 °C/min, and held for 4 min for a total run time of 17 min
Flow Rate:	1.0 mL/min
Injection Temp:	250 °C

MS Conditions

Manifold Temp:	50 °C
Transfer line Temp:	250 °C
Ion Trap Temp:	200 °C

MS/MS Parameters

Time (min)	Precursor Ion (*m/z*)	Excitation Storage (*m/z*)	Excitation Amplitude (V)	Product Ion (*m/z*)	High Mass Ejection
4-6.5	212	90	1.10	170-215	12
	217	90	1.10	170-220	12

Figure 2. Varian 240-MS Ion Trap mass spectrometer with 450-GC and CombiPAL AutoSampler.

Results and Discussion

TCA was determined quantitatively using TCA-d5 as the internal standard with the parameters described above using EI/MS/MS. The matrix effects were eliminated using MS/MS mode, which provided excellent qualitative and quantitative analysis of TCA and internal standard TCA-d5 (Figure 3).

In this method, the precursor ion selected for TCA-d5 is *m/z* 217. The major product ions for this precursor ion are *m/z* 199 and 197. The precursor ion used for TCA is *m/z* 212. The main product ion of TCA is *m/z* 197. Since these two precursor ions at *m/z* 217 and 212 are present in two different MS/MS channels, the common product ion at *m/z* 197 from TCA and TCA- d5 will not interfere with each other as shown in Figure 4.

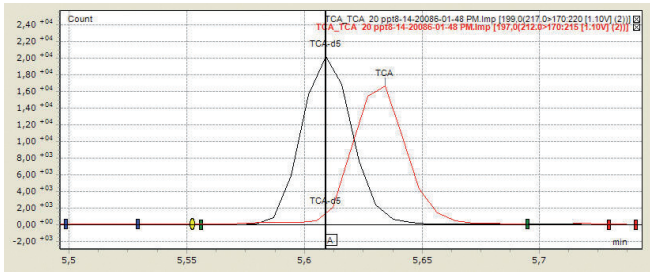

Figure 3. Multiple reaction monitoring (MRM) of TCA and TCA-d5 at 20 ppt.

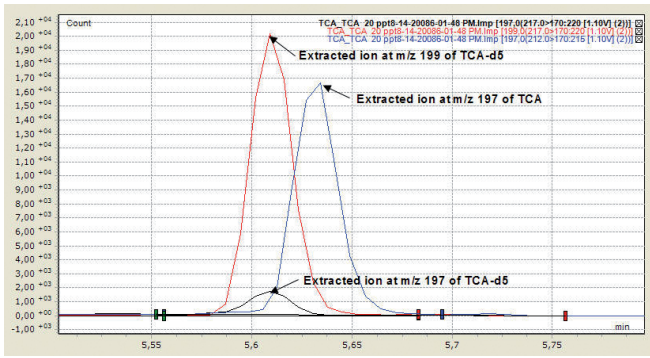

Figure 4. Extracted ion chromatogram (EIC) of TCA-d5 and TCA. Common product ion at *m/z* 197 originates from two separate MS/MS channels so that no interference in quantification is observed.

Under these conditions, TCA can be determined as low as 0.1 ppt in white wine with a signal-to-noise ratio of 5 (peak-to-peak) (Figure 5). The calibration coefficient and relative standard deviation of TCA was determined at concentrations ranging from 0.1 to 20 ppt in white wine. The r^2 and %RSD of TCA are 0.99997 and 10.2%, respectively (Figure 6). MS/MS spectra and chromatograms of TCA at 1 ppt and TCA-d5 at 20 ppt are shown in Figure 7. MS/MS offers unique selectivity and specificity with positive identification of TCA at 1 ppt in the presence of the internal standard.

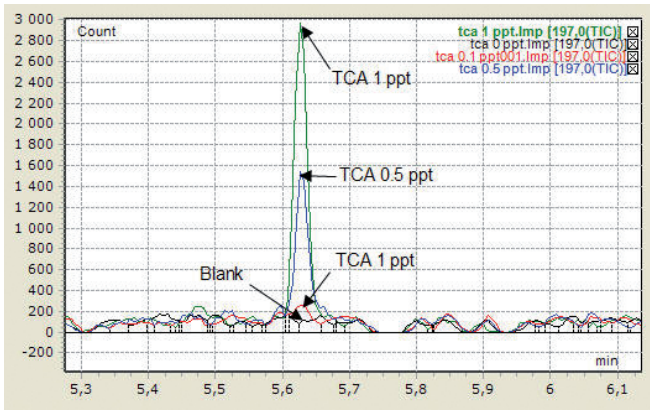

Figure 5. EIC of TCA at *m/z* 197 at concentrations of 0.0 (blank), 0.1, 0.5 and 1 ppt. S/N ratio of TCA at 0.1 ppt is 5 (peak-to-peak).

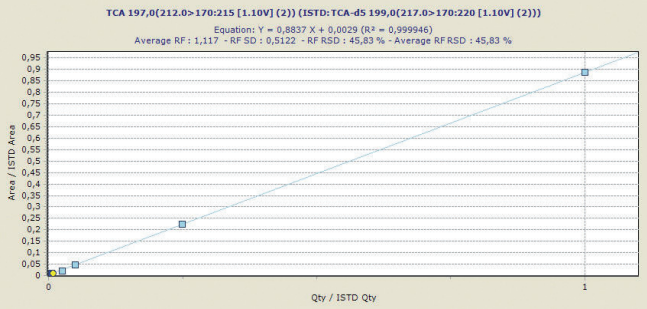

Figure 6. Calibration curve of TCA at concentrations of 0.1, 0.5, 1, 5 and 20 ppt with TCA-d5 at 20 ppt as internal standard.

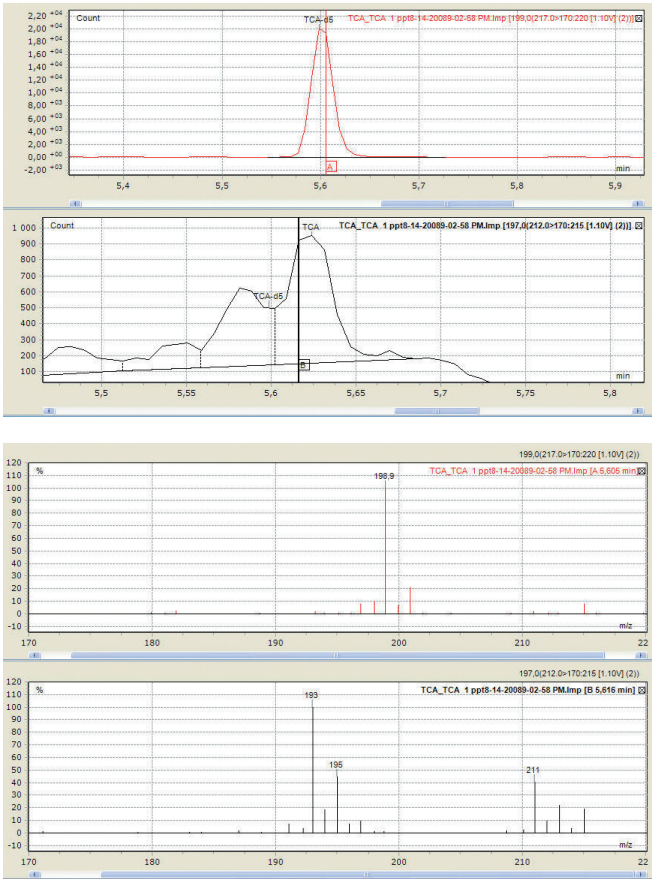

Figure 6. Top: Extracted ion MS/MS product ion chromatograms for TCA (1 ppt) and TCA-d5 (20 ppt). Bottom: Corresponding product ion spectra for TCA (1A) and TCA-d5 (2A).

Conclusion

An EI/MS/MS method was developed for the determination of 2, 4, 6-trichloroanisole (TCA) in white wine, quantitatively as low as 0.1 ppt. This level is nearly ten times below the human olfactory threshold. The MS/MS method eliminates the matrix effects commonly observed in SPME extractions of wine, providing lower detection levels and greater confidence in the data. The method is highly sensitive, selective, and easily automated for routine high throughput quantitative analysis.

References

1. Rochkind-Dubinsky, M. L., Sayler, G. S. and Blackburn, J. W. (1987) *Microbiological Decomposition of Chlorinated Aromatic Compounds*. Marcel Dekker, Inc., New York, NY, USA.

2. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD, USA. http://toxnet.nlm.nih.gov/

3. Evens, T.J., Butzke, C.E. and Ebeler, S.E. (1997) Analysis of 2, 4, 6-trichloroanisole in wines using solid-phase microextraction coupled to gas chromatography-mass spectrometry. J. Chromatogr. A., 786(2): 293-8.

4. Hoffmann, A., Sponholz, W. R., David, F. and Sandra, P. (2000) Corkiness in Wine-Trace Analysis of 2, 4, 6-Trichloroanisole by Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC/MS. Appnote 3/2000. Gerstel Global Analytical Solutions, Mülheim, Germany.

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